JOURNAL OF EXPERIMENTAL BOTANY

AN OFFICIAL ORGAN OF THE
SOCIETY FOR
EXPERIMENTAL BIOLOGY

VOLUME II

OXFORD
AT THE CLARENDON PRESS
1951

Oxford University Press, Amen House, London E.C.4
GLASGOW NEW YORK TORONTO MELBOURNE WELLINGTON
BOMBAY CALCUTTA MADRAS CAPE TOWN
Geoffrey Cumberlege, Publisher to the University

PRINTED IN GREAT BRITAIN
AT THE UNIVERSITY PRESS, OXFORD
BY CHARLES BATEY, PRINTER TO THE UNIVERSITY

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W. W. Schwabe, Factors controlling Flowers in the Chrysanthemum, II (pp. 223–37), Plate II, Figs. 8 and 9 should be transposed.

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The Haustorial System of *Viscum album*

D. THODAY

WITH 2 PLATES

SUMMARY

1. The strands of *Viscum album* in the rind of the host are bilateral, even at the apex. Most commonly the tip is triangular in section, with the broad side outwards; some are broader and more massive, others more slender and less clearly dorsiventral. The forwardly directed outer and lateral marginal cells grow out into elongated hyphal or club-shaped cells with swollen laminated walls. In advance of them a space opens in the host tissues, filled with mucilage probably produced in part by the *Viscum* but with host cell-contents lying in it. A small-celled meristem, naked, lies on the inner side, towards the host wood. Between this and the hyphal fringe is a transitional region where the cells are larger and more elongated in a direction obliquely forwards and inwards. A procambium strand near the inner side can be traced nearly to the small-celled tissue. The whole apex can be interpreted as a terminal meristem, the different parts of which respond to a gradient of conditions, from the host cambium, in contact with which a sinker is eventually initiated, to the cortex, where growth is aggressive and the digestive hyphal cells are formed: a few of these may become separated, but many are incorporated in the outer tissues of the strand.

2. On germination of the 'seed', the tip of the hypocotyl responds to contact by unequal tangential growth of the sides, to flatten the convex meristematic face against the host and to produce a dome-shaped holdfast. Copious secretion of a viscous sealing fluid, giving the reactions of cutin, follows near the margin of the holdfast and the epidermal cells of the face grow out into papillae which adhere to the host surface. Further growth of the walls of the holdfast dome, the growth of new papillae, thrusting outwards underneath, and the collapse of a subcortical zone of cells, which may have a contractile effect as in the contractile roots of *Oxalis* spp., result in the progressive lifting and disintegration of the host periderm till a slit is opened by which the primary haustorium penetrates. The latter has the form of a narrow oval wedge; in it a median plate of xylem is differentiated. In the host its behaviour depends on its orientation and on the conditions it meets. In bottle cork it remains a simple wedge. Its edge is slightly papillate and probably secretes a pectic enzyme. It divides to pass strands of fibres in its inward passage to the host cambium, and it forms the cortical strands as lateral outgrowths, running chiefly parallel to the axis of the host branch. Xylem is initiated as a median plate or, in narrower branches, as a central strand.

3. For the formation of the holdfast, close contact and firm pressure with exclusion of air are required. In response to yielding pressure the face of the hypocotyl tip bulges and although papillae arise a holdfast is not formed. In the absence of a contact stimulus, as in air, on agar, or on a slippery glass surface, the face grows out and no papillae are formed.

4. It is clear that in these haustorial organs structure and behaviour are closely interconnected and highly specialized in relation to the functions of the system.

Journ. of Experimental Botany, Vol. II, No. 1.

INTRODUCTION

This re-examination of the haustorial system of *Viscum album* followed from a previous study of a very reduced and specialized member of the Loranthaceae, *Arceuthobium pusillum* (Thoday and Johnson, 1930), which spreads in the tissues of its host as filaments of cells. Multicellular strands are formed from these filaments by longitudinal division of the cells, which are anomalous in structure as well as in origin. The corresponding strands of *Viscum album* are not so reduced, but are also anomalous in structure; although they possess features which some authors have regarded as root-like, opinions have differed regarding their morphological nature.

Initially (as in *Arceuthobium*) the xylem is nearly central. The apical growth is difficult to interpret. Superficial cells near the tip seem to become loosened laterally from connexion with the underlying tissue and form obtuse club-shaped forward-growing papillae of some length, the walls of which are much swollen. The strand thus appears to end in a more or less irregular brush, which is embedded in a 'viscid slime' (Solms-Laubach, 1868). These outstanding characteristics have been observed by all authors who have dissected out the delicate young strands (Pitra, 1861; Hartig, 1876; Melchior, 1923). They are bright green except near the tips where the green fades gradually to a pale yellowish apex without chlorophyll.

Melchior (1923), the latest to attempt elucidation of their structure and mode of growth, with modern methods at his disposal, remarked on the difficulty the investigation presents. There is general agreement that the hair-like cells are the end cells of rows that can be followed back a little distance. Pitra (1861) regarded these terminal cells as the youngest, presumably, therefore, the initials. Eichler (1868), too, concluded that no root-cap is present. Solms-Laubach at first (1868) regarded the hair-like cells as constituting a sort of root-cap, and found a dome of small actively dividing cells behind it (Pl. XXXVIII, Fig. 1) corresponding to the sub-apical meristem of a root; but later (1875) he doubted the root-cap and inclined to Pitra's view. Melchior (1923), however, concluded that there is a sort of transverse meristem which in front cuts off cap-cells that elongate and loosen, and ultimately die and are sloughed off, and behind add to the length of the strand.

Scrutiny of the published evidence leaves the reader unable to decide for himself regarding the facts. Are the end cells growing forward actively and cutting off daughter cells behind them? Is the growing-point superficial or not? Melchior's evidence is not conclusive. His only figure of an apex (p. 68) represents a small tapering one; but he remarks that they are not all of this type. Some have the appearance of a broad obtuse brush. It seems to be taken for granted, too, that the tip is radially symmetrical, an assumption which is not justified, as the sequel will show.

Some interest attaches to the solution of this morphological problem from the comparative, evolutionary point of view. *Viscum album* is an outlier extending to the northernmost limit in Europe of a family which is primarily tropical; it might therefore be expected to show specialized rather than primitive characters. In the genus *Loranthus* itself, the descriptions of *L. europaeus* suggest a plastic haustorial tissue in which it is difficult to see any root-like features. *Viscum capense*, judging from a preliminary examination of material from Mauritius, for which I am indebted to Dr. H. Evans, is still more amorphous within the host. A higher degree of morphological organization in *V. album* might therefore, as in *Arceuthobium pusillum*, be derived rather than primitive. Whatever conclusion might be reached from the point of view of comparative morphology, a study of the development and structural differentiation of such haustorial organs is of considerable interest for causal morphology. As previously emphasized in reference to *Arceuthobium*, the endophytic system develops from embryonic tissue embedded in living host tissues and therefore under conditions and influences which may resemble those governing normal differentiation of tissues in the host itself. In so far as this is the case, the resulting structure will express a system of relations not wholly inherent within the parasite. It is from this point of view that the investigation was begun. It started with the apical growth of the cortical strands, and has extended to the primary haustorium and the early development of the seedling as a whole.

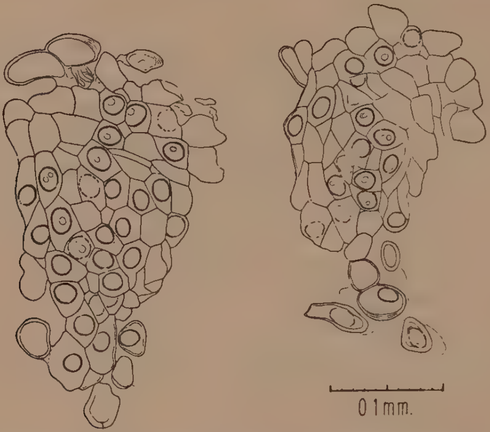
Supplies of material were generously provided by Mr. J. W. Williams of Skenfrith and Sir Richard Leighton of Loton Hall. Mr. H. Jenkinson informed me of a successful technique for sowing the berries and provided material for the College gardens. Thanks are due also to Miss A. J. Davey and Miss B. M. Hitching for assistance rendered.

PART I. THE APEX OF THE CORTICAL STRANDS

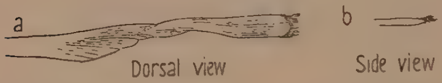
Accounts hitherto published give neither an exact description of the commonest forms of apices nor an adequate picture of the range of forms exhibited. Hartig (1876), Solms-Laubach (1868), and Melchior (1923) all describe them as tapering to a brush-like tip where the cells elongate and tend to separate. In apple and hawthorn, however, the tip is seldom symmetrical round its own axis, but is commonly like a wedge or thick blade, radially placed with reference to the host twig. The end view, and similarly sections transverse to the host just behind the very end, are then roughly triangular, with the apex of the triangle towards the host cambium (Text-fig. 1). A more slenderly tapering form is, however, often met with in spring on hawthorn. More massive tips occur, either in the form of larger radial wedges, or sometimes still broader tangentially and relatively flattened in plane radial to the host tissues (Text-fig. 2). By far the larger number show evident dorsiventrality in profile: and it will be convenient to refer to the side towards the host cortex as dorsal, that towards the host wood as ventral.

The tip is embedded in and partly held together by a dense gelatinous material. In part this is derived from the swelling walls of elongated superficial cells, as evidenced sometimes by striations, in part from disorganized host cells. Evidences of dissolution are common enough in the form of crystals and other host cell-contents lying loose in the mucilage.

For a millimetre or two in front of the tip a radial slit opens in the host phloem. Three factors probably contribute to this: these are (i) the solvent action of enzymes secreted by the tip, especially pectase attacking the middle lamella; (ii) tissue tensions in the host phloem, which must tend, as cambial activity proceeds, to widen such a gap once formed; (iii) growth in thickness of the strand itself behind the tip. The strand usually thickens at least fast enough to keep the space filled; but one case was observed in which this was not so, and host callus had filled a gap on each side of the strand.



TEXT-FIG. 1. Cortical strands of *Viscum album* cut near apex transversely to the host stem: host cambium below.



TEXT-FIG. 2. *a*, dorsal view, i.e. from cortical side, of cortical strand in poplar, of broad form, showing localization of renewed extension growth and papillate fringe at the tip. *b*, side view of apical part, ventral surface below.

As Melchior has pointed out, the cells are smaller just behind the brush-like tips and increase in size farther back. The enlargement of cells which is thus indicated must result in the tip being pushed forward; but it advances into mucilage-filled space, or, in so far as there is friction, the mucilage provides lubrication.

Some of the outer elongated cells, as Melchior observed, do show degenerate contents. In most of them, however, the protoplasm and large nuclei look healthy and vigorous. It is not their invariable or even their usual fate to be sloughed off, for they can be found farther back, many still alive and incorporated in the outermost layer of cells, a few with their forward ends bent back as if by friction with the host tissues.

In the less common slender tapering form of apex the cell rows which end in the elongated cells of the brush are often directed straight forward. In other forms, however, a very general and striking feature, when the tip is viewed laterally, is the evident curvature of the cell rows towards the host wood.

The absence of radial symmetry in the apex itself and slight sinuosities of the strand make orientation for sectioning, whether *in situ*, or even more when isolated, a task of considerable difficulty and uncertainty. Yet from what has been said it follows that there is only one plane of section which can be expected to yield a clear view of the construction of the apical region, viz. a median longitudinal section in the dorsiventral plane, radial to the host twig.

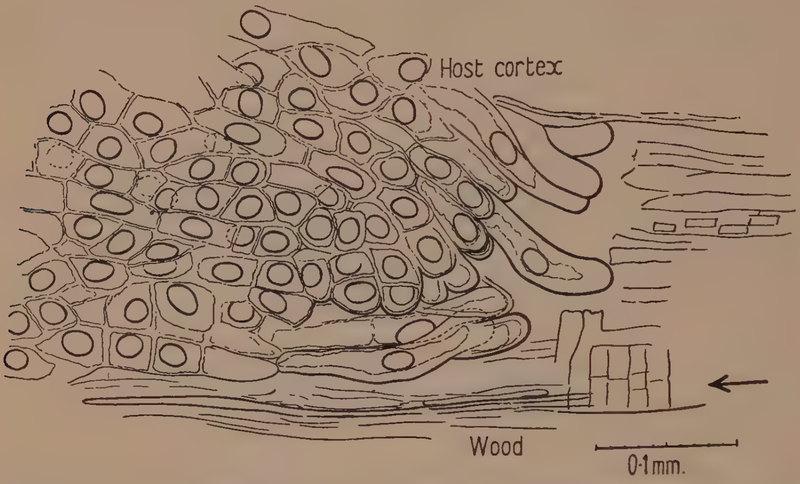
The methods which have helped the elucidation of the structure have been:

(1) sectioning with the freezing microtome at 25μ of selected apices: staining the sections in cotton blue in lactic acid was specially useful;
(2) cutting freehand sections, in which orientation was more under control;
(3) clearing whole apices in potash or in lactophenol. Dehydration contracted the cell walls so much that embedding in paraffin wax for serial sections was rejected.

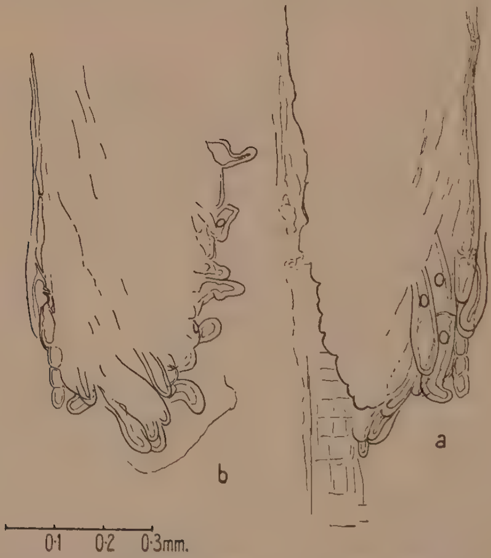
Several apices have been drawn from both sides under the camera lucida before being sectioned with the freezing microtome. In this way proper seriation and orientation of the sections and their registration with one another were facilitated. The greatest source of uncertainty and difficulty lay here in the fragility of the sections. This was very largely met when the apices were embedded in strong gelatine, hardened after cooling in strong formalin. The material could then be more accurately oriented on the microtome, the sections were more easily handled, the apical brush held together better, and discontinuities due to curvature of the strand itself were bridged.

A section obtained by the first method is represented in Text-fig. 3. It is a nearly median section of an apex dissected from an apple branch in July. It gives an impression of a ventrally curving naked apex, the lateral and frontal cells of which grow out into the characteristic thick-walled elongated cells of the brush, while the lower central part pushes down towards the host wood.

Some doubt was possible regarding the exact orientation of this section and its relation to adjacent ones; but Text-figs. 4 and 5 leave no doubt that the interpretation is essentially correct. They are from an apex obtained in January from hawthorn. Text-figs. 4*a* and *b* show its external appearance before sectioning, as seen from opposite sides. Text-fig. 5*a* represents the section most nearly median, with the outline of the whole apex (Text-fig. 4*b*) indicated by thick broken lines, for reference; Text-fig. 5*b* represents the section next but one behind. The forward slope is marked by the characteristic elongated cells. Behind is a mass of small cells in which it is not easy to make out any regularity of arrangement. Certain features emerge, however, on close examination. Immediately behind the elongated forward cells there are evidences of cell-division parallel to the frontal surface; the rows of cells in Text-fig. 5*b* show, too, a general curvature parallel to this surface. In the more median section (Text-fig. 5*a*) a second feature obscures this arrangement: the continuation of the vascular strand is indicated by the shape and



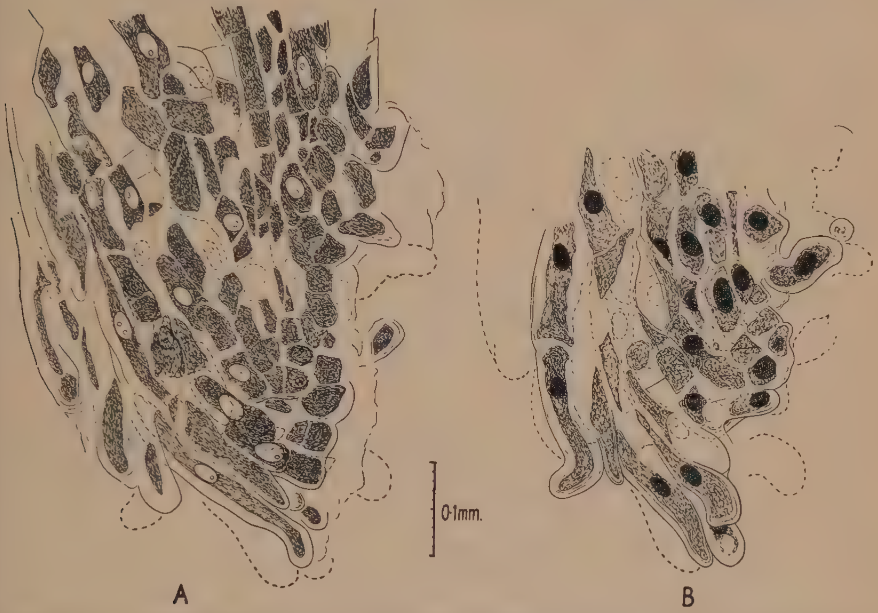
TEXT-FIG. 3. Nearly median longitudinal section of cortical strand, radial to the host branch (apple, July). The arrow indicates the position of the host cambium.



TEXT-FIG. 4, *a* and *b*. Apex of a cortical strand from hawthorn, viewed whole from opposite sides: ventral, i.e. inner, surface to left in *a*.

arrangement of cells in the formation of which the cell-divisions appear to have cut across the downward curvature.

It is clear that the apical meristem is asymmetrical. By its growth it must lift the most superficial of the elongated cells of the fringe up on to the dorsal surface (and displace some also to the flanks by lateral thickening). New fringe cells grow out from below the older ones, or sometimes thrust out between them as they become separated by the growth in thickness (Text-fig. 4*a*). The elongation of the superficial cells takes place in part while they are still in close organic union with the actively dividing cells just below them. Two main directions of the division walls can be distinguished, one longitudinal, following the frontal curve, the other transverse to this direction; but the longitudinal walls are often oblique, giving spindle-shaped cells. The smallest isodiametric cells seem to be located in line with the direction of propagation of the procambial strand.



Text-fig. 5. Longitudinal sections of the cortical strand shown in Text-fig. 4, radial to the host branch: section *a* is approximately median.

It seems highly probable that this form of apex represents the summer and winter condition when the tip of the strand is making contact with the wood to establish a new sinker, or is dormant. In the spring, apices of a more slender tapering form are common: they probably represent a phase of more rapid elongation. The alternation of the two phases might account for the intermittent formation of sinkers, unattached propagating loops being formed in spring while the host cambium is active, the strand later reaching the wood and establishing a new graft. The strand drawn in Text-fig. 3 shows evidence of local renewal of activity at an earlier stage of its growth.

Examination of 2-year-old infections (on hawthorn) makes its clear that a second sinker is sometimes formed in one season.

The host cambium is stimulated to prolonged, or renewed, or greater activity when the apex of the parasite strand reaches it and, by interrupting it, makes contact with the host wood. Such hypertrophy is often conspicuous following the penetration of the primary haustorium during the first season as far as the cambium, especially on small branches, as it results in a marked swelling at the point of infection. This is especially obvious on *Tamarix* (Plate I, Figs. 1 and 2). Here the cambial activity is evidently prolonged beyond its normal duration. The hypertrophy is strictly localized except in the basipetal direction. Below 2-year-old infections on hawthorn, basipetal extension of the hypertrophy is usually very evident for several centimetres. This is suggestive in relation to the predominantly basipetal conduction of growth-hormones in plants.

The observations here recorded lead to the general conclusion that the apical meristem is terminal and that the peculiar features of symmetry, structure, and development are related to the special situation and function of the organ.

The anatomical development behind the apex has not yet been fully elucidated. The first formed xylem is probably to be regarded as occupying an equivalent position to that in *Arceuthobium*,[1] but as the organ is dorsiventral from an early stage the morphological centre is displaced. Later growth is slight ventrally, greatest dorsally. In some sinkers traces of xylem strands have been found which may be continuations of those of the original cortical strands; if so, continuity must be maintained during the intercalary growth of the sinker. These xylem strands are marked by spiral vessels, contrasting with the reticulate tracheides in the rest of the mature sinker tissue.[2] It would be interesting to follow the development of these 'primary' xylem strands in relation to the initiation of sinkers and the seasonal cycle.

PART II. THE PRIMARY HAUSTORIUM

The cortical strands first arise as branches from the primary haustorium. To examine their mode of origin and follow the development of the primary haustorium itself it was necessary to examine seedlings in process of establishment. These were first obtained by sowings on apple, hawthorn, poplar, and a variety of other host plants. Later, seedlings were obtained on non-living substrata in the laboratory, and various devices were used in the attempt to obtain straight seedlings for anatomical examination and to study their reactions.

It soon became evident that previous descriptions were inadequate. The impression gleaned from the literature was that of a conical peg thrust into the host from below the attachment disk or holdfast, by which the tip of the seedling secures itself. Löffler (1923) claims to have based his account on a thorough examination of many young seedlings and mentions several features not previously recorded; but even he seems to imply a radial symmetry. A casual reference by Tubeuf (1923, p. 536) to the primary sinker as 'ein senkerartige Gewebekeil' is probably to be given a mechanical rather than an exact morphological interpretation. It would appear that previous investigators must have oriented their sections relative to the host twig exclusively either transversely or radially. Had they cut tangential sections at an early stage in the establishment of the seedling, they could not have missed the obvious bilateral symmetry.

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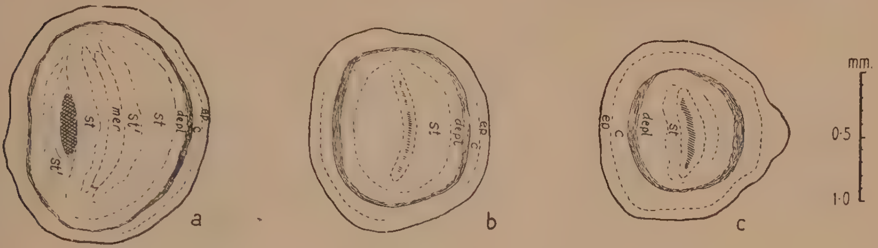
[1] Melchior (1923) says definitely that in the young strand the xylem is central.

[2] Pitra (1861) gave a different account and it remains to be determined whether the xylem of the cortical strand can invariably be traced into the sinker.



Text-fig. 6. Sections through the holdfast of a young seedling hardly yet penetrating into the host, cut at different levels parallel to the surface of the host: *ep*, epidermis; *c*, cortex; *mer*, meristem; *st*, starch-containing cells; *st'*, less starch; *depl*, depleted tissue, the outer zone collapsing as if crushed. In *a* the plane of section was below the surface of the holdfast in the cross-hatched area; in *b* and *c* a median plate of xylem has differentiated (shaded).

Text-fig. 6 represents in outline the main features of sections through the holdfast cut parallel to the surface of attachment, of a seedling which had barely begun to penetrate into the host. Among other features to which reference will be made later, they show the narrow band of xylem (shaded) extending along the major axis surrounded by small-celled tissue still more or less meristematic and continuous with the meristematic growing edge of the primary haustorium which the lowest section almost includes.

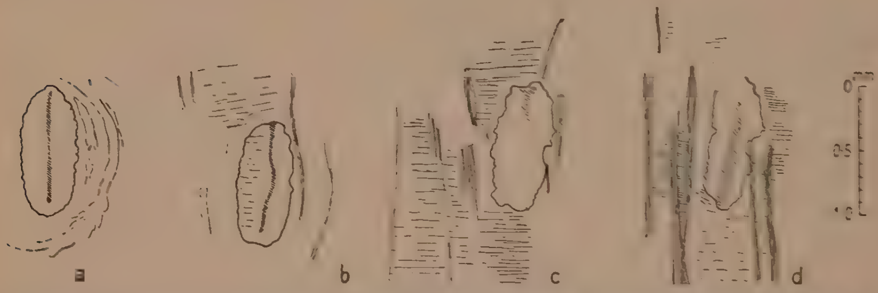
Text-fig. 7 shows a similar tangential series cutting across a primary haustorium which has already reached the host phloem. No xylem has yet been differentiated in it, but a median plate of still meristematic tissue is indicated by shading, continuous with a broader meristematic region at the growing edge of the haustorium.

It is clear that careful orientation of sections is essential to the success of any attempt to elucidate not only the structure of the haustorium itself but also that of the holdfast at all stages. The direction of the long axis of the haustorium cannot, however, be determined with any certainty by inspection of the intact seedling.

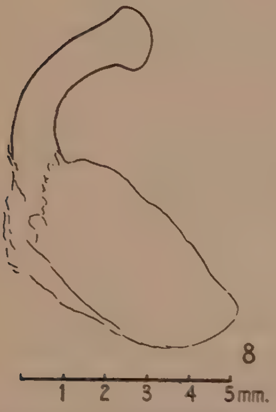
It was in part this difficulty that led to closer study of the structure of the seedling, in all stages of development, and of the reactions whereby it contrives to establish itself. These reactions proved to be unexpectedly complex.

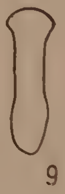
In the process of establishment a succession of phenomena may be distinguished:

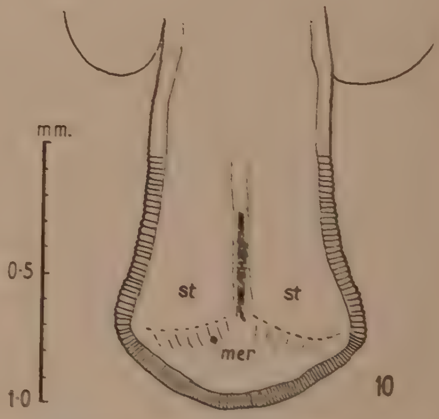
(*a*) As is well known, the hypocotyl responds negatively to both gravity and light. These two responses, of which the phototropic response is dominant, bring the tip into contact with the substratum.



TEXT-FIG. 7. Sections, tangential to host, across a young primary haustorium. In *a*, the outline of the base of the holdfast is partly traceable; median plate of meristem indicated by shading; in *b*, indications of growth expansion at right angles to the meristematic plate; host fibres represented in *c* and *d*.





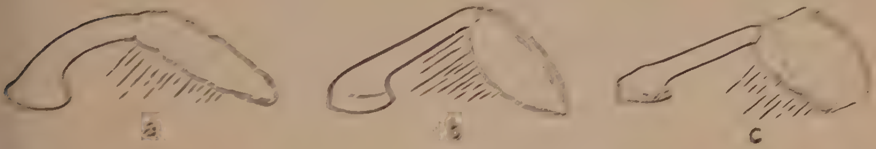


TEXT-FIG. 8. Germinating 'seed': hypocotyl protruding, showing expanded tip with convex surface.

TEXT-FIG. 9. Embryo, for comparison with Text-fig. 8, dissected from ungerminated 'seed'.

TEXT-FIG. 10. Tip of hypocotyl just protruding from 'seed', in median longitudinal section: *mer*, meristematic; *st*, starch; *x*, xylem.

(*b*) In relation to the next stage it should be observed that the diameter of the hypocotyl in the unattached seedling increases a little towards the blunt convex tip (Text-fig. 8). It will be convenient in referring to this terminal knob to distinguish (Text-fig. 10) the *shoulder*, with normally cutinized epidermis, and the initially convex *face*, with soft moist appearance, where the epidermis is still meristematic (the interior of the tip is also meristematic). Contact of any part of the face with the substratum acts as a stimulus, inducing growth of the outer, cortical tissues of the shoulder. This results in a flattening of the face. Commonly one side makes contact first: growth is then unequal, the side farthest from the point of contact growing fastest. The face is thus both flattened and brought squarely against the substratum. The result is a more or less dome-shaped holdfast with closely fitting base. Between the holdfast and the rest of the hypocotyl there is commonly a sharp bend, which, it may be observed in passing, is an obstacle in the way of anatomical investigation.



TEXT-FIG. 11. Seedlings on slips of cork over agar. *a* was removed from the tube after formation of holdfast had begun, so that the viscin hardened. *b* and *c* remained in the tubes plugged with cotton wool; the viscin did not harden and the 'seeds' were lifted by the growth of the hypocotyl.

(*c*) Two further responses also follow contact:

(1) the face secretes copiously a viscous substance, which gives the reactions of cutin: it is fluid, for it soaks into porous substrata like filter-paper or cambric;

(2) the epidermal cells of the face, especially near the margin, form papillae, which adhere closely to the surface of the substratum.

Eventually the fluid exposed at the margins of the holdfast begins to harden; but meanwhile the growth of the cortex of the holdfast continues for a time so that it widens its area of contact. The fluid may therefore act as a lubricant, the hypocotyl meanwhile keeping the holdfast in contact with the substratum by its growth curvature in the first phase of the process.

The normal course of these developments is dependent on definite conditions, and when one or other of these conditions is not provided the resulting deviations from the normal throw further light on the complex of co-ordinated reactions.

1. *The seed must be firmly attached.* In some cultures made under moist conditions which prevented the proper hardening of the viscin, the growth of the hypocotyl lifted the 'seed'. The hypocotyl appeared then to elongate and straighten, except next to the 'seed' and next to the holdfast where it was sharply bent (Text-fig. 11). This indicates that after contact is established the hypocotyl is no longer responding by curvature, but thrusting.

2. *Firm contact and increasing pressure are normally required.*

(*a*) Seedlings failing to make contact show a forward expansion of the shiny face, without enlargement at the shoulder to form a normal holdfast. Examples are shown in Text-figs. 12, 13, and 14.

(*b*) Contact with smooth glass has not in these experiments provided the necessary conditions: the shiny, lubricated face slips on it and there is no difference then from tips in air. (Tubeuf, however, figures normal holdfasts on glass: possibly a drier atmosphere would harden the secretion, reduce its lubricant quality sufficiently, and so secure fixation.)

(*c*) Light contact is insufficient. Seedlings to which thin slips of cork were attached showed no response (Text-figs. 15, 16). It is probable that the viscous secretion prevents effective contact.

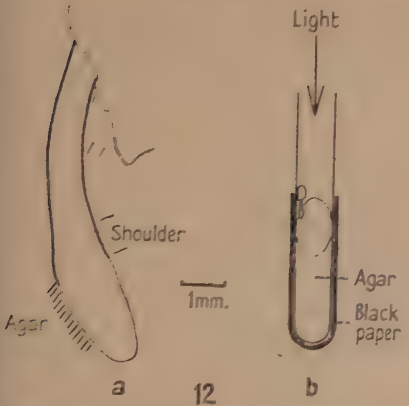
(*d*) Constant moderate pressure can induce the formation of papillae, but such swelling as does occur is insufficient to flatten the face properly against the yielding surface. Instead, the face tends to bulge as in unstimulated tips. This is illustrated in Text-fig. 17. The hypocotyl of this seedling had grown obliquely upwards. A light wooden lever, balanced on a central pivot, was arranged obliquely with one end touching the tip (Text-fig. 17*a*). To the upper side immediately above the seedling a small weight was added, so that the hypocotyl lifted it in its growth but could not increase the pressure as in a normal attachment. (On the right, where the face had grown out more, the formation of papillæ had been intermittent.)

When contact has been properly established the further developments are perhaps even more remarkable.

1. The widening and lifting of the dome, partly due to general cortical expansion, partly to some extension at its margin, pulls the papillae which have already attached themselves to the host surface upwards and outwards. In the case of the seedling on hawthorn shown in Text-fig. 18 the following sequence is to be inferred. The outermost detachable layer of host tissue is torn off and carried up by the adhering papillae. This happens on either side of the future haustorium and begins the formation of a slit in the protective surface of the host. A succession of further papillae then attach themselves to layer after layer of host periderm, till eventually the slit exposes living host cells. In the section illustrated, which was stained in Sudan red, three such successive layers of cork could still be traced, displaced and partly fragmented.

2. The progressive lifting of these papillae and of the epidermis from which they arise is not solely due to the growth of the dome, for with it are associated the collapse of cells in a definite zone just within the cortex of the dome (Text-fig. 18*c*) and growth of the new papillae underneath, some of which expand bladder-like, while thrusting under the cork layer already separated.

It is possible that the collapsing cells play a quasi-active part similar to that played in the contractile roots of *Oxalis* spp. by transverse zones of cells which lose their protoplasm: in this case the withdrawal of their sap to other



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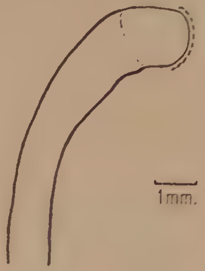

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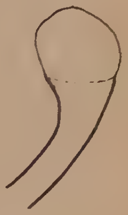

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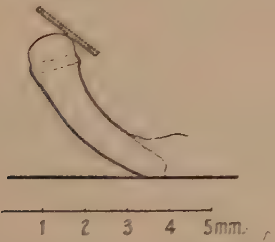
Text-fig. 12*a*. Seedling on agar slope, showing protrusion of face: no holdfast was formed; 12*b* shows its position in the tube, darkened below by a sheath of black paper.

Text-figs. 13 and 14. Seedlings on agar in plugged tubes, showing protrusion of face and no holdfast.



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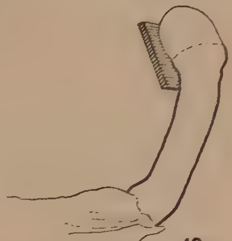

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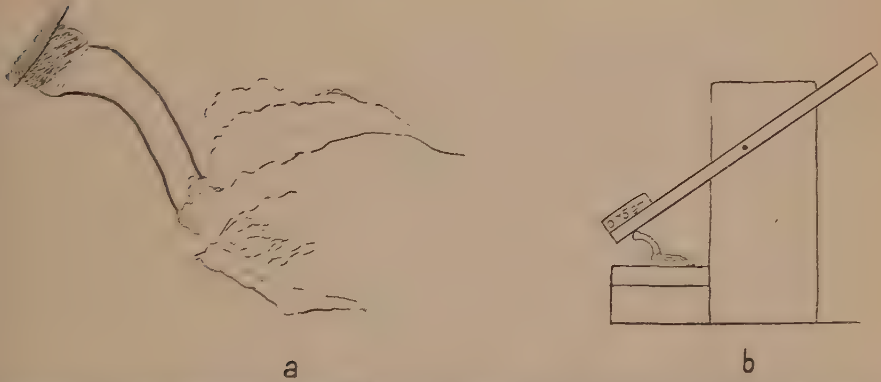
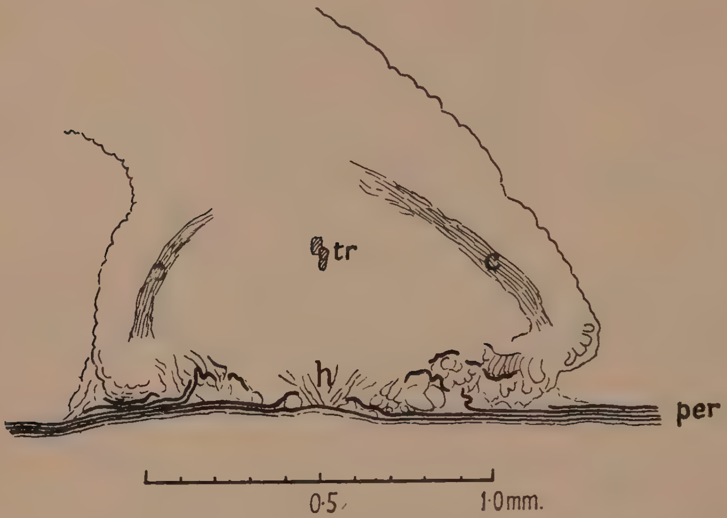
Text-figs. 15 and 16. Seedlings with slips of cork adhering to sticky face, which bulges as when no effective contact is made.



Text-fig. 17. Seedling with upwardly directed hypocotyl made to lift a weight: the arrangement is shown in *b*; *a* shows the bulging of the face, which, however, responds to the contact by the formation of papillae—note zonation.

parts results in a reduced pressure or even a cohesion tension, which pulls together the still turgid zones that alternate with the collapsing ones, so shortening the root (Thoday, 1926).

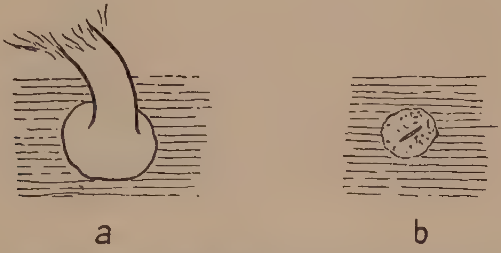


Text-fig. 18. Longitudinal section, approximately median, at right angles to the long axis of the primary haustorium, *h*, which is just beginning to protrude: *per*, periderm—the successive layers of cork are shown as thick lines, traceable as broken and/or lifted portions inwards towards *h*; *tr*, tracheides; *c*, collapsed tissue.

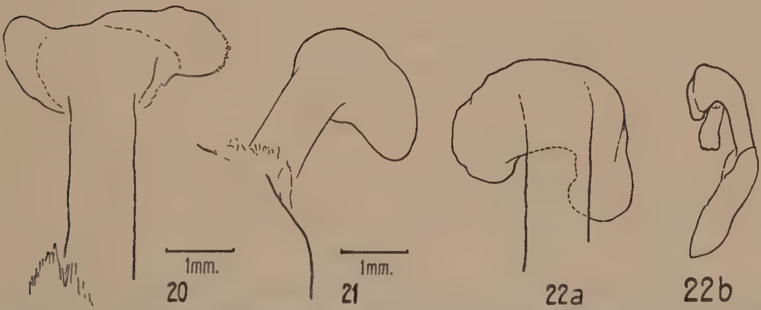
That a similar process may occur in the mistletoe seedling is indicated by the observation that in some cases the dome itself becomes depressed towards the host surface. There does not appear to be any other obvious explanation of this. If the base were attached to an unyielding surface, active collapse of the cells under the dome would draw the walls down towards the base. It would also reinforce the thrust of the haustorial wedge.

3. While the host surface is being attacked in this way the meristematic region in the middle of the holdfast becomes active. There is at first no well-defined apex. The haustorium is delimited from the surrounding tissue by progressive vacuolation of the surrounding cells. When the haustorial wedge has entered the host the forward edge is meristematic, sometimes with a fringe of papillae: the exact mode of its early development has still to be satisfactorily elucidated. Such sections as have been obtained leave it an open question whether the epidermis ever takes part in its production, but it is probably hypodermal (cf. Plate II, Fig. 8).

Tubeuf describes another mode of growth, particularly characteristic of seedlings in moss or peat, where the tip of the seedling grows out into root-like extensions which not infrequently branch dichotomously (loc. cit., pp. 534–5 and Figs. 116, 117). He figures examples of some length with more than one bifurcation. Similar organs are also stated to occur on irregular surfaces, as in the case of the Pine mistletoe. He calls them 'epicortical roots' and says they are able to penetrate the tissues of the host at the grooves between leaf-bases.



Text-fig. 19. Seedling on slip of cork over agar: *a*, from above, showing typical holdfast; *b*, from below the cork, showing oval patch where cork has been dissolved; the tip of the seedling is seen to be papillate, with fragments of cork adhering; the edge of the haustorial wedge is emerging (seen as a green line in the actual material).



Text-fig. 20. Seedling on vertical surface of wood, showing incomplete formation of holdfast and outgrowths on both flanks: papillae visible on right.

Text-fig. 21. Seedling on cambric supported by glass in vertical position: outgrowth on one flank directed obliquely backwards (downwards).

Text-fig. 22. Seedling on cambric, supported as before: outgrowths on both flanks, both directed backwards; *b* shows side view on a smaller scale.

Structures that are probably to be compared with these 'epicortical roots' have been obtained in the present investigations on two types of substrata, namely, on wood and on cambric supported by glass.

In a number of cases the resistance of a wood surface enabled the seedlings to form normal attachment disks, though the haustoria could not penetrate it: in thrusting they merely lifted the holdfast from the surface. In other cases, however, a normal holdfast was not formed; instead the face protruded, as in seedlings growing freely. Some of these grew further to form on one or both flanks cylindrical outgrowths (Text-fig. 20).

Examples strikingly similar to these were obtained on cambric (Text-figs. 21 and 22). In these the crumpling of the cambric between the tip and the seed showed that after the first contact a continuance of the curvature of the hypocotyl had drawn the tip backwards. The cylindrical outgrowths on the flanks were directed backwards towards the 'seed'.

Two connected factors might explain the aberrant behaviour of these seedlings. (1) The atmosphere was probably moist enough to keep the viscin soft and so prevent the development of sufficient pressure for the formation of a normal holdfast. (2) The curving hypocotyl moved the tip over the surface of the substratum and the resulting friction was sufficient to stimulate it to continue growth. On cambric, this growth was in the direction of movement and of resistance.

It would appear from a consideration of the evidence that an essential condition for outgrowth of the face is exposure to air. The normal holdfast with its copious secretion excludes air and so would inhibit general expansion; differentiation and penetration follows. An irregular surface does not provide the level base required (Tubeuf, loc. cit., p. 534) and air is not excluded. Cambric soaks up the secretion.

In the absence of contact of any sort, growth may soon cease, though Tubeuf describes rather larger expansions than those here described for freely growing seedlings, with some examples even showing early bifurcation (loc. cit., Fig. 117*a*, 1).

Friction, on the other hand, stimulates the organ to continue growth—even such slight friction as moss would provide. Tubeuf's 'epicortical roots' creeping over the surface of the host are examples of this response, which includes both the continuance of growth and a thigmotropic reaction.

When such organs meet obstacles they may circumvent them, sometimes possibly by bifurcation, to judge from one of Tubeuf's figures (loc. cit., Fig. 117*a*: 3–5). If, however, they enter a groove that blocks their passage, such as the axil of a leaf-base of *Pinus*, the conditions are provided for penetration. No details of the process of penetration in such a case are available, but Tubeuf's figures of resulting attachments show no indication of a special holdfast.

If the tip of an 'epicortical root', which has arisen directly from the tip of a seedling, can penetrate the host, the question arises whether the assumption of an endogenous primary haustorium is justified and whether the whole of the original meristematic tip should be regarded as potential haustorium, including the face as well as the meristematic mass behind it. What has been regarded as endogenous haustorium would then be merely that part of it in which the meristematic condition persists when differentiation has begun.

Even after primary establishment has been achieved, growth of the proximal part of the primary haustorium often lifts the old holdfast from the host surface and epicortical strands may grow out from the exposed tissue just below it. This could be interpreted as the result of exposure to air. It would imply also a tendency to localization of growth and the production of cylindrical outgrowths, of which the cortical strands from the primary haustorium are other examples.

Further growth of the primary haustorium within the living tissues of the host varies with the nature of the host. In poplar and in apple it enlarges more rapidly and branches more freely than in hawthorn. In a piece of bottle-cork it has a simple structure, almost unbranched (Plate I, Fig. 3).

Two examples that were worked out in detail suggest that the *orientation* of the wedge in relation to the host also has an effect. Models of the haustoria reconstructed to scale from sections are shown in Plate I, Figs. 4 and 5. In the first, on poplar, the edge of the wedge happened to lie parallel to the length of the host twig: branches had arisen from the margins of the wedge and grew chiefly upwards and downwards. In the second, on hawthorn, the wedge had entered transversely: the branches were again longitudinal, but from the faces of the wedge, and were broad and irregular in form.

Besides producing lateral outgrowths the main haustorial wedge may also divide to by-pass obstacles such as strands of fibres (cf. Plate I, Fig. 4, top). It therefore appears to be a very plastic organ.

Differentiation of xylem occurs in the median plane of the wedge. It begins near the top of the holdfast dome at an early stage (cf. Fig. 18, *tr*), in continuity with a small knot of tracheides which represents the common terminus of the xylem strands of two cotyledonary bundles which gradually converge in the hypocotyl and finally fuse. The xylem of the haustorium continues as a median plate until branching occurs. Xylem is then differentiated conformably in each segment—in cylindrical branches as a central strand.

EXPERIMENTS ON THE PROCESS OF PENETRATION

It is clear that growth pressure is an important factor in the penetration of the host by the haustorium. The holdfast when properly established provides the necessary purchase so that the growth pressure may be effective. Its active contraction, as explained above, may provide an additional thrust. There remains the question how far the entry is facilitated by solvent action.

To obtain light on this question a number of sowings were made on various substrata, including bottle cork, filter-paper, and fine cambric supported on cork or glass, and thin slips of cork on glass or agar. Conclusive evidence of solvent action on cellulose was not obtained. Papillae penetrated singly or in groups between warp and woof of the cambric. The absorption of the viscous secretion by cambric and especially by filter-paper appeared to lead to exhaustion of the reserves of the seedling and abortion of the haustorium.

Definite evidence was, however, obtained of disintegration of cork. Large holes appeared in thin slips of cork and the papillate under side (face) of the holdfast could be seen and photographed through the glass (Plate II, Fig. 6). The remains of the cork appeared as brown fragments scattered over the surface. A definite line marked the position of the emerging haustorial wedge (Text-fig. 19).

It seems highly probable, therefore, that a secretion from the face has a solvent action on the pectic middle lamellae of the cork. It is probable also that the haustorial wedge has a similar action. When it is penetrating thick pieces of bottle cork, the cork just in front of it shows in section signs of altered staining reactions, e.g. to ruthenium red, as the middle lamella is swollen slightly. At a very early stage of penetration a definite groove was observed in the cork, which did not look like a result of compression (Pl. II, Figs. 7 and 8).

MORPHOLOGICAL COMMENTS

Some of the simpler features of the holdfast of *Viscum album* are shown by the attachment organs of *Ampelopsis* spp. in varying degree. In *A. Veitchii* tendrils are negatively geotropic. Their tips, swollen before contact, enlarge after contact. The epidermis responds by formation of papillae and secretion of mucilage (Langerken, 1885; cf. Goebel, *Organography*, i. 268). In *Viscum album*, however, the holdfast of the seedling is clearly a far more highly specialized organ in which tropic and other physiological responses, development, and structure are co-ordinated and adapted in remarkable detail to securing effective attachment and assisting the penetration of the haustorium into the host.

The primary haustorium and the cortical strands, while exhibiting a high degree of plasticity, behave consistently and follow a definite pattern in their growth and differentiation which is neither that of a root nor that of a shoot. Comparative study of other Loranthaceae may provide evidence with regard to the evolutionary origin of the haustorial system; but whether or not it arose as an organ *sui generis*, its unique character requires that it should be so regarded now, and no useful purpose would appear to be served by attempting to homologize it with plant members belonging to the recognized categories.

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D. THODAY—PLATE I

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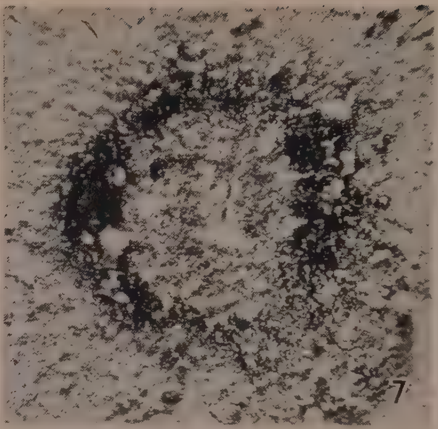

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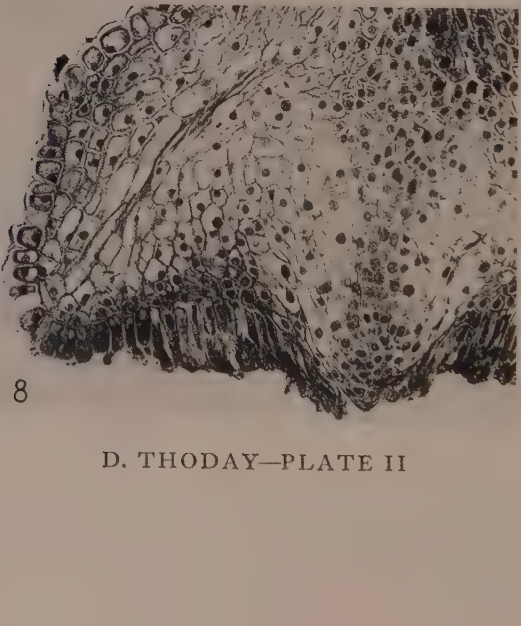

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D. THODAY—PLATE II

DESCRIPTION OF PLATES

Fig. 1. Tamarisk branch with boss resulting from establishment of a seedling of *Viscum album*. The hypocotyl has been removed (? eaten off) just above the holdfast.

Fig. 2. Part of the specimen shown in Fig. 1, cut radially through the *Viscum* seedling. Stimulation of wood formation is very marked in the near neighbourhood of the infection and has extended basipetally beyond the limits of the section.

Fig. 3. Plasticine model of the primary haustorium of a seedling which had penetrated a piece of bottle cork. The model was moulded to scale from a series of sections. Note the simple form, like a wedge, with only a short rudimentary branch near the holdfast.

Fig. 4. Wax model, from a series of sections cut with a freezing microtome, of the primary haustorium of a seedling on poplar. The long axis of the wedge was oriented longitudinally. Cortical strands have formed from the lateral margins of the wedge. The main part of the wedge, penetrating radially inwards, has divided into three portions, avoiding strands of fibres, and one of them has already become embedded in host wood (the embedded portion shown darker).

Fig. 5. Wax model, similarly constructed, of the primary haustorium of a seedling on hawthorn. The long axis of the wedge was transversely oriented. Branches have grown out from the faces of the wedge, i.e. in the longitudinal direction, and are broad, flat, and irregular, in contrast to those shown in Fig. 4.

Fig. 6. Slip of cork, which has been perforated by a *Viscum* haustorium, photographed from the reverse side through the supporting glass slide. The surface of the haustorium is papillate. Around it the cork has been lifted away from the glass.

Fig. 7. Photograph of the surface of a piece of cork on which a holdfast had been formed and which the primary haustorium was just beginning to penetrate. It has made a groove in the cork. Note that the viscid secretion (appearing dark) was abundant under the peripheral part of the holdfast, but is not evident under the central part.

Fig. 8. A nearly median longitudinal section of the seedling removed from the cork of Fig. 7, showing the papillate lower epidermis and the emerging haustorium. Note the zone of collapsed cells within the cortex, above the most elongated epidermal cells of the face of the holdfast.

The Submicroscopic Organization of the Cell Wall in Conifer Tracheides and Wood Fibres

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AND

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WITH 2 PLATES

INTRODUCTION

THE cell-wall organization of conifer tracheides has been the subject of considerable controversy during the past fourteen years (Kerr and Bailey, 1934; Preston, 1934, 1939; Bailey and Berkeley, 1942) and it is the purpose of this paper to examine the further evidence now available and to present what the writers consider to be the solution of the problem. A preliminary note on this work has already been published elsewhere (Wardrop and Preston, 1947). The opposing views have been expressed clearly in earlier papers (Bailey and Berkeley, 1942; Preston, 1946), but for convenience a résumé is given here. The controversy has centred around the observation that, when viewed in transverse section between crossed nicols, the secondary wall shows marked optical heterogeneity in that two bright layers can be observed: a narrow outer layer and a narrow inner layer, separated by a central dark layer which in the late wood cells is thicker than its adjacent bright layers. The cell walls of wood fibres behave similarly, and a transverse section viewed between crossed nicols of *Nothofagus cunninghamii* and *Picea* sp. is shown in Plate I, Figs. 1 and 2.

On the basis of observations such as the run of striations in surface view (Bailey and Berkeley, 1942), the orientation of iodine crystals grown in the wall (Bailey and Vestal, 1937*a*), and features associated with fungal attack (Bailey and Vestal, 1937*b*), Bailey and his collaborators attributed the optical heterogeneity described above to differences in orientation of the cellulose micelles in the three layers distinguishable in the secondary wall. Thus in the dark middle layer the micelles were supposed to be oriented in a steep spiral, while those in the inner and outer layers were thought to lie more or less transversely (Kerr and Bailey, 1934), or, in later papers, at angles between 45° and 90° to the longitudinal axis (Bailey and Vestal, 1937*a*). The case where the micelles of the outer layer are oriented transversely and those with the middle layer longitudinally is shown diagrammatically in Text-fig. 1 *a*.

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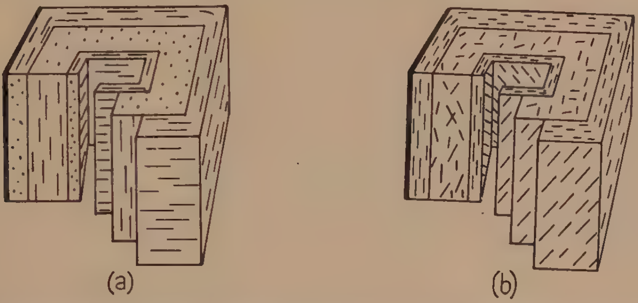
[1] The bulk of the work described here was carried out in the Botany Department, The University of Leeds, during the tenure of a Commonwealth Science and Industry Endowment Fund Research Scholarship.

On the other hand, Preston (1934), in a paper presented in the same year as the first of the Bailey series, used X-ray and optical methods to support the view figured earlier by Frey-Wyssling (1926; see also Jaccard and Frey, 1928) that the micelles in the wall of the tracheide lie more or less in the same orientation throughout the whole wall thickness, and later criticized the evidence obtained from swollen or otherwise distorted walls. From the evidence it was suggested that the optical heterogeneity was to be explained by a pronounced difference in the angular dispersion between the layers (Text-fig. 1 *b*) and, later, that any wall layers with a transverse micellar orientation could be present only to an extent so small as to give no record on the X-ray diagrams of conifer wood, and to cause no disturbance of the optical properties of the wall in surface view (Preston, 1947). Again, the X-ray evidence apparently supporting the crossed fibrillar structure brought forward by Bailey and Berkeley (1942) was disposed of as an error in interpretation (Preston, 1946), and it seemed at that time that the case was at last clear, as far as the physical evidence went.



TEXT-FIG. 1. Two models originally prepared for the wall structure of conifer tracheides. The lines represent the run of the micelles. (*a*) After Bailey. (*b*) After Preston.

There remained, however, some unsatisfactory features. Thus it was shown by Wardrop (1946), in work not previously published, that if single cell walls of tracheides or wood fibres from abnormal tissues with thick walls, such as compression wood of gymnosperms or tension wood of angiosperms, were crushed and stained with congo red, at least two striation directions could be detected, the dichroic properties of which showed that they did, in fact, represent two different micellear directions differing in orientation by 15° to 45° depending on the material observed (Wardrop and Dadswell, 1948, 1950). While these observations were carried out on abnormal tissues and did not reveal the presence of any striations approaching the transverse, they did clearly suggest the need for further examination of the cell walls from normal tissues.

Further evidence brought forward (Wardrop, 1946) concerned the study of the birefringence of the cell wall of different cell types. Thus the observations of Preston (1946) showed that the birefringence of the outer layer in transverse section varied between 0·004 and 0·015, and this was interpreted to indicate that the outer layer was inclined in a steep spiral. Now when a comparative examination was made of the birefringence in transverse section of the outer layer of the secondary wall in the fibres and vessels of *Nothofagus cunninghamii* it was found to be similar to that of the outer layer of the secondary wall in *Picea* (Table I) and to lie within, although near the maximum of, the range of values for the birefringence of the outer layer of the secondary wall in various conifers as observed by Preston (1946). However, the vessels of *Nothofagus* possess a ring type of wall organization in that the micelles lie approximately transverse to the longitudinal vessel axis, so that in this case at least there was evidence of a cell with transverse micellar orientation of which the birefringence would suggest that the micelles were oriented in a steep spiral. The fact that the birefringence of the outer layer was low did not, of itself, necessarily indicate that a steep micellar spiral was actually present as suggested by Preston. In view of the similar magnitude of birefringence in the vessels and outer layers of the walls of fibres from *Nothofagus* and the outer layer of the tracheides of *Picea*, similar dichroic behaviour might be expected. This behaviour is illustrated in thin delignified sections stained with congo red of *Nothofagus* and *Picea* in Plate I (Figs. 3 and 4). It will be clear, however, that, since birefringence is governed by many factors, mere correspondence of the magnitude of birefringence or similar dichroic behaviour in different cell types does not guarantee identity of structure.

TABLE I

The birefringence of various tissue elements observed in transverse section

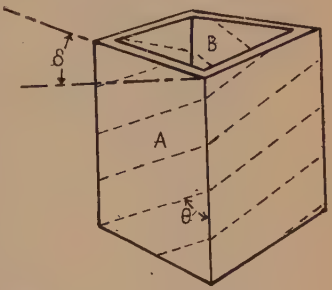
Tissue element	Birefringence
Tracheides *Picea* sp. (outer layer of secondary wall) . . .	0·010–0·014
Tracheides (various species) (outer layer secondary wall) Preston (1946)	0·004–0·015
Fibres (*Nothofagus cunninghamii*) (outer layer secondary wall) . .	0·009–0·012
Vessels (*Nothofagus cunninghamii*)	0·011–0·015

Considerations such as these made the physical evidence appear less conclusive and it became very desirable that a series of observations should be designed sufficiently critical to distinguish clearly between the two hypotheses; it is with such an experiment that the present paper is concerned.

METHOD AND RESULTS

Of the two hypotheses presented above, only that of Kerr and Bailey (1934) demands any net change in orientation between the wall layers. Now it had long been evident that if the birefringence of the various layers in transverse section of a cell could be studied as the section was tilted about a line perpendicular to the direction of observation and to one wall surface, a clear-cut distinction between the two hypotheses could be made. Thus as any set of cellulose chains approached a position perpendicular to the direction of observation, passed through it, and receded, then the birefringence should pass through a maximum. A different angular position of the maximum in the different layers would then imply a different chain orientation. Difficulties associated with the technique and the nature of the material made this impossible, but it occurred to us that the same observations could be made in another way. Thus, if a series of sections were cut at increasing angles from the transverse, then a series of observations on changes in birefringence of the various cell-wall layers could be made analogous to those which could have been made on a tilting cell. In Text-fig. 2 a single spiral inclined at an angle θ to the longitudinal cell axis is represented. It will be clear that, for the wall *A*, as the angle of section δ increases the birefringence will increase, reaching a maximum when the section is parallel to the cellulose chains (angle of section $= 90° - \theta$) and then decrease again, while for wall *B* the birefringence will decrease to a minimum at an angle of section θ and then increase again.

If, then, the whole of the secondary wall is uniform in micellar orientation as suggested by Preston (Text-fig. 1 *b*), the birefringence of all layers should show a maximum at the same angle of section. On the other hand, if the wall consists of two or more coaxial spirals as suggested by Kerr and Bailey (Text-fig. 1 *a*), their maximum birefringences should be found at different angles of sectioning for the different cell-wall layers (i.e. the bright and dark layers of Plate I, Figs. 1 and 2). Among the disadvantages of this method as compared with the progressive tilting of a single cell it should be pointed out first that, since different parts of different cells are being examined in different sections, an average birefringence for many cells has to be obtained; secondly, it is imperative that all the tracheides should have spirals of the same sign; and lastly, it is preferable that outer and central layers should have spirals of the same sign even if the inclination of the spirals is indeed different. These conditions seem to be met in a specimen of *Picea* sp. and *Nothofagus cunninghamii* Oerst (Hook), and a series of observations on sections cut from each was therefore made. In both cases it was found preferable to deal with the tangential walls, so that, commencing with a transverse section, sections were cut at decreasing angles to the radial longitudinal plane. Now since in the specimens used the different cells each had a spiral micellar organization of the same sign, it is clear that in examining the birefringence of the outer bright layers of two adjacent cells, in effect a wall of type *A* would be adjacent to a wall of type *B*, Text-fig. 2, so that as the outer bright layer increases in birefringence with increasing angle of section (Text-fig. 2) in the one wall, so the adjacent bright layer of the next cell would tend to decrease in birefringence. As it was found easier to measure changes in birefringence passing through a maximum rather than through a minimum, walls with micellar orientation with respect to the plane of sectioning of type *A*, Text-fig. 2, were chosen in each cell examined. This was done by keeping constant the



TEXT-FIG. 2. Diagrammatic representation of a section of a cell, the wall being built up of one molecular spiral whose tilt is indicated by the dotted lines.

orientation of the blocks of wood used so that, for example, the tangential wall nearer the centre of the stem of each cell in each section was always observed.

Phase difference determinations, ϕ, were made in sodium light using a de Sénarmont compensator. The section thickness, d, was subsequently determined by turning the cells on their sides and measuring directly with an eyepiece micrometer. The birefringence could then be calculated from the relation

$$n_\gamma - n_\alpha = \frac{0{\cdot}59\phi}{360d}.$$

TABLE II

The birefringence of tracheides from Picea *sp. at different angles of section*

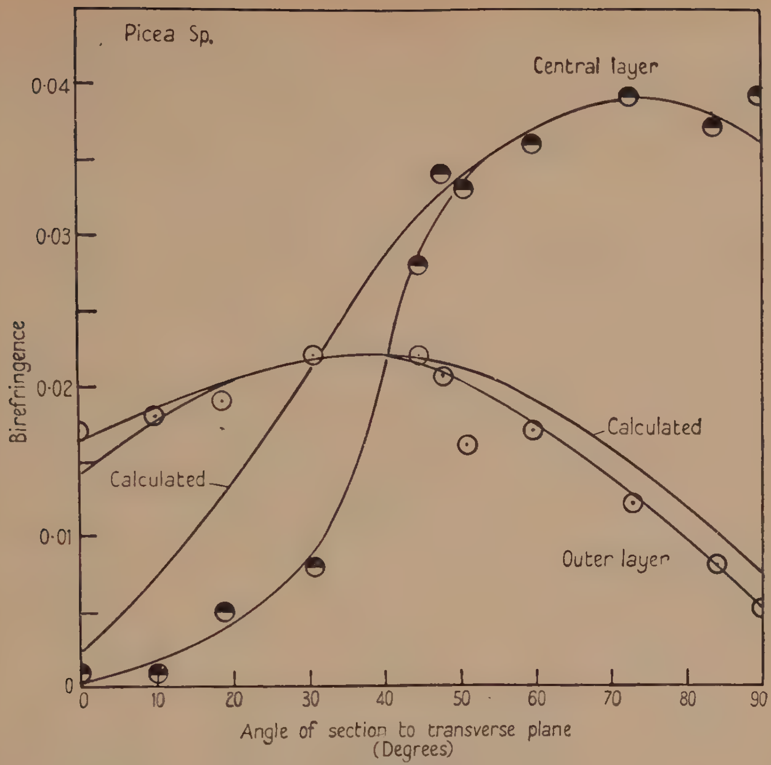
Angle of section to transverse plane (degrees)	Birefringence outer layer		Birefringence central layer	
	Observed	Calculated	Observed	Calculated
0	0·017	0·014	0·001	0·003
10	0·018	0·017	0·001	0·007
19	0·019	0·020	0·005	0·014
31	0·022	0·022	0·008	0·022
38	—	0·022	—	—
45	0·022	0·022	0·028	0·031
48	0·018	0·022	0·034	0·033
51	0·016	0·021	0·033	0·033
60	0·017	0·019	0·036	0·037
73	0·012	0·015	0·039	0·039
84	0·008	0·010	0·037	0·038
90	0·005	0·008	0·039	0·036

TABLE III

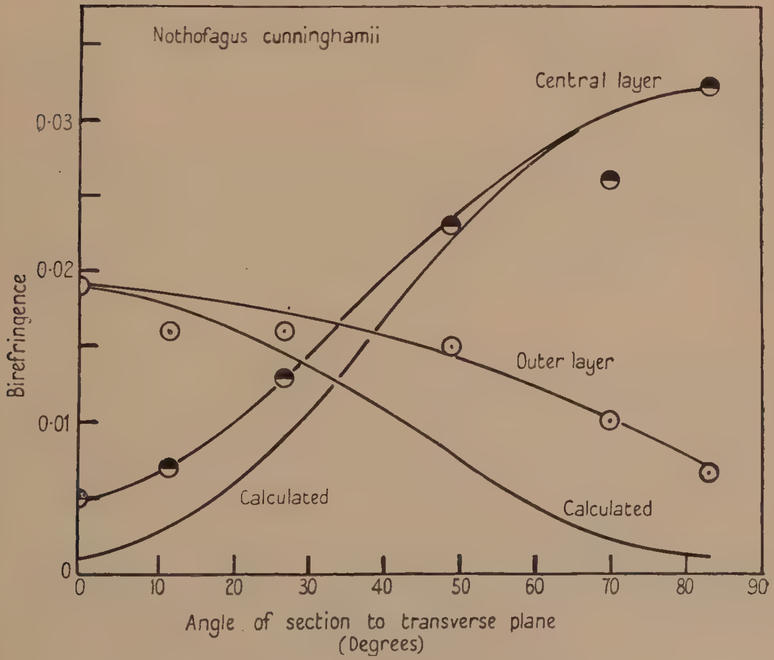
The birefringence of fibres from Nothofagus cunninghamii *at different angles of section*

Angle of section to transverse plane (degrees)	Birefringence outer layer		Birefringence central layer	
	Observed	Calculated	Observed	Calculated
0	0·019	0·019	0·005	0·001
12	0·016	0·018	0·007	0·003
27	0·016	0·015	0·013	0·009
49	0·015	0·008	0·025	0·022
70	0·010	0·002	0·026	0·031
83	0·009	0·001	0·032	0·032

The birefringence measurements of outer and inner layers of the cell wall at different angles of section of the tracheides of *Picea* and the fibres of *Nothofagus* are given in Tables II and III respectively and are illustrated graphically in Text-figs. 3 and 4. From the maximum birefringence of each layer, i.e. the birefringence when viewed normal to the length of the constituent micelles, it is possible to calculate the birefringence value in any other position, and it is of interest to compare the calculated and observed values. Thus, if the



Text-fig. 3. For explanation see text.

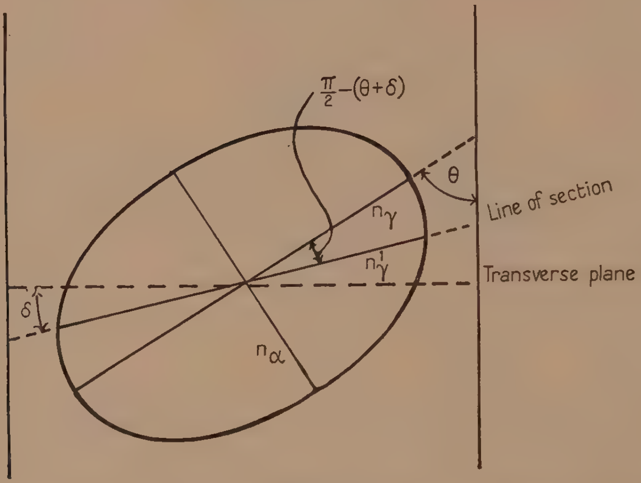


Text-fig. 4. For explanation see text.

maximum birefringence is denoted as $n_\gamma - n_\alpha$ (Text-fig. 5), then at any angle of section δ to the transverse plane the birefringence should be

$$n^1_\gamma - n_\alpha = \frac{n_\gamma\, n_\alpha}{[n_\alpha^2 + (n_\gamma^2 - n_\alpha^2)\cos^2(\theta+\delta)]^{\frac{1}{2}}} - n_\alpha.$$

Taking n_α as 1·53 as is usual with cellulose, and knowing $n_\gamma - n_\alpha$, then n^1_γ can be calculated for each layer at each angle of section so that $n^1_\gamma - n_\alpha$ can be calculated for any value of δ. The corresponding curves are also presented in Text-figs. 3 and 4.



TEXT-FIG. 5. The orientation of the index ellipsoid as seen in face view of the wall. For details see text.

X-ray examination of specimens matched with those sectioned was made, using CuK_α radiation and a specimen-film distance of 3 cm. The X-ray diagram of *Picea* is shown in Plate II, Fig. 2, and of *Nothofagus* in Plate II, Fig. 1. From the spread of the equatorial arcs the micellar spiral angle ϕ corresponds to 20° in *Picea* and 10° in *Nothofagus*.

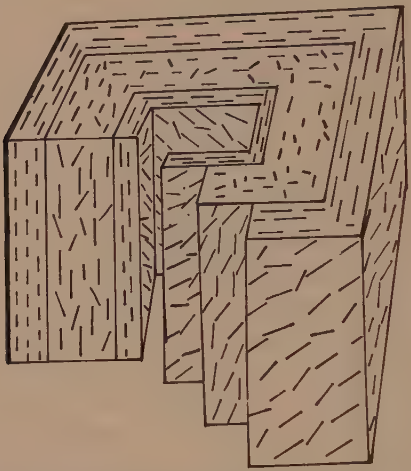
DISCUSSION

From Text-Figs. 3 and 4 it is at once clear that the maxima in the birefringence curves for the central and outer layers of both conifer tracheides and wood fibres do not coincide. In *Picea* the micelles in the outer layer make an angle of some 50° to the longitudinal tracheide axis and those of the central layer are inclined at an angle of 20°. In *Nothofagus* it is somewhat difficult to determine the precise angle of the outer layer as no clear maximum can be detected, but the angle must be between 90° and 70° to the longitudinal cell axis, and in any case is certainly much greater than that of the central layer in which the micelles, as can be seen from Text-fig. 4, are inclined at less than 10° to the longitudinal cell axis. These considerations would then seem to settle the major point at issue in that they indicate that one difference between the different layers of the secondary wall (Plate I, Figs. 1 and 2) is in micellar spiral angle as suggested by Kerr and Bailey (1934), and that, irrespective of other factors operating, the optical heterogeneity of the wall must in a large degree depend upon this difference in organization.

A comparison of the calculated and observed values of birefringence, Tables II and III, reveal a considerable divergence, especially in the regions of low birefringence where the experimental error is most likely to be great. It is, however, unlikely that this divergence is entirely due to experimental error, and it can probably be attributed to the dispersion of the micelles about the preferred direction of orientation. The effect of angular dispersion of the micelles is particularly clear in the case of the outer layer of the fibres of *Nothofagus*. Again, some angular dispersion is indicated in the divergence of the observed and calculated values for the central layer of the wall, which may be related to the observation made originally by Preston (1939), and repeated during our present work, that the major extinction position of a wall in transverse section is sometimes inclined to the wall surface. On account of the smallness of the birefringence measured and the possibility of relatively large experimental errors, it is impossible from the above curves to assess exactly the influence of angular dispersion on the birefringence of the cell wall. Consideration of the values of the maximum birefringence in the various layers, however, suggests strongly a difference in angular dispersion between the outer and central layers as well as the difference in chain direction. Thus the maximum birefringence of the outer layer in *Picea* is 0·022 and in *Nothofagus* 0·019. This can be compared with the value which would be expected if in fact no angular dispersion occurred, provided the percentage cellulose content of the wall layer concerned could be estimated. Although this is not known, it has been reported (Ritter and Fleck, 1926; Allsopp and Misra, 1940) that the average percentage cellulose content of the whole wall is approximately 60 per cent. both for late wood, where the central layer is well developed, and for the early wood where it is not. Now, assuming no angular dispersion, the birefringence of the outer layer would equal the observed value of 0·022 in *Picea* only if the cellulose content of this layer were about 38 per cent. This value would appear to be low compared with those usually found by total analysis of the material and would therefore suggest the possibility of micellar angular dispersion in this layer of the wall; but until the exact cellulose content of the different wall layers can be established no final conclusion can be reached upon this point. This might eventually be achieved using the methods employed by Lange (1948). For the central layer a figure of 60 per cent. cellulose content agrees satisfactorily with the observed birefringence.

Thus it may be concluded that the outer and central layers in the material studied here differ in at least two respects, i.e. the net orientation of the cellulose micelles is different and there is in addition a difference in angular dispersion, in cellulose content, or in both, which reduces the intrinsic birefringence of the outer layer to an unforeseeably low figure (Text-fig. 6).

The main point at issue may thus be regarded as settled, but there remain several features of particular interest to discuss. A comparison of the micellar spiral angles determined for the central layer from birefringence determinations with that obtained from the X-ray diagrams shows remarkable agreement. This makes it certain that the X-ray diagram refers almost exclusively to the central, dark layer. The failure of inner and outer layers to contribute effectively to the X-ray diagram is still somewhat difficult of explanation. Obviously the lower cellulose content here may be involved, or the higher angular dispersion and recent work by the writers (Preston and Wardrop, 1949) lends support to this view. Work carried out in this laboratory on the fibres of sisal (Preston and Middlebrook, 1949) is also of interest from this point of view. In these fibres it is clear that the outer layer does give strong characteristic reflections up to the time at which the central layer is laid down. Once this layer (which is dark between crossed nicols in transverse section) is present, and the fibre becomes mature, then this central layer alone contributes to the X-ray diagram, and the arcs which would be expected from the outer layer are apparently missing. It is not to be supposed that the cellulose content per cell layer has changed during this deposition, and it seems most likely, therefore, that the disappearance of the diagram is due to an increase in angular dispersion, consequent, perhaps, on lignification, which would cause a spreading of the arcs. Some evidence in support of the possibility exists in the observations made during the present investigation that delignification causes an increase in birefringence of the outer layers, and in an earlier observation that delignification of coconut fibres causes a decrease in angular dispersion of the cellulose remaining as determined in the X-ray diagram (Preston and Allsopp, 1939). If it is supposed that this effect is reversible, then it is reasonable to



TEXT-FIG. 6. Prepared model for the wall structure of conifer tracheides. The outer and inner layers differ in net fibrillar orientation, though not so markedly as in Text-fig. 1 *a*, and in angular dispersion as in Text-Fig. 1 *b*.

suppose that lignification would be accompanied by an increase in micellar dispersion. The increase in layer thickness of cell walls during lignification, such as is frequently reported in the literature (see, e.g., Preston, 1941), would lend further support to such a view. Some estimate of the increase in dispersion consequent upon lignification can be made from the observation that in *Nothofagus* delignification causes an increase of 15 per cent. in the birefringence of the outer layer in transverse section. This can be shown to indicate a change in dispersion of the order of 15°, so that in untreated cells the angular dispersion must be at least ±15°.

The lack of appreciable effect of the outer layer on the properties of the whole wall in surface view, such as the major extinction position, is certainly due to similar factors. Thus it was shown some years ago (Preston, 1934), and has since been demonstrated in more specific terms (Preston, 1949), that in conifer tracheides of length L and breadth B in which the spiral angle is θ the relation

$$L/B = K \cot \theta,$$

or, in the more recent work,

$$L = K_0 - K_L \cot \theta_s,$$

holds with some precision, where the K's are constants and θ_s is the spiral angle for a standard breadth. The former of these two relations was developed on the assumption of the type of wall organization shown in Text-fig. 1 *b*, but in view of the preceding discussion it is apparent that this relation applies only to the central layer, as has actually since been established (Preston, 1948). More recently it has also been found that a similar relationship also holds with the outer layers of the secondary walls themselves (Preston and Wardrop, 1949).

Thus while the evidence demands the operation of some factor such as angular dispersion within the separate wall layers, and there is little evidence of any layer with chains lying strictly transversely, or even approximately so in conifer tracheides, the optical heterogeneity of the wall does depend primarily on a difference in the micellar spiral angle. In conclusion it would appear justified to say that the observations described above do effectively distinguish between the two hitherto divergent hypotheses concerning the structure of tracheide walls, and to point out that this has been achieved through the use of physical approaches only to untreated wall material. They would therefore appear to offer a sound foundation upon which to consider other problems of wall structure, as well as those involved in the investigation of cell-wall deposition and orientation which form the major subject of study in this field at the present time.

ACKNOWLEDGEMENTS

For assistance with sectioning the writers wish to express their indebtedness to the Division of Forest Products, C.S.I.R., Melbourne, Australia, and to Mr. R. Metcalfe of the Royal Botanic Gardens, Kew, Surrey. They are also

indebted to Dr. H. E. Dadswell, Forest Products Division, C.S.I.R., Australia, in whose laboratory the preliminary observations described in the Introduction were carried out.

SUMMARY

The controversy concerning the details of structure in the walls of conifer tracheides has been resolved by a study of the birefringence observed in sections cut at various angles to the radial longitudinal plane. It has been demonstrated that the optical heterogeneity observed in transverse section is traceable to two factors: (i) The outer layer of each tracheide is composed of cellulose chains lying in a rather flat spiral, though not transverse, making an angle of some 50°, on an average, to cell length; in the thicker central layer the spiral is much steeper, with an angle of some 20°. (ii) At the same time the birefringence of both layers is reduced in virtue of both high lignin content and high angular dispersion, the dispersion being considerably greater in the outer layer.

It is presumed that this high angular dispersion in the outer layer is responsible for the lack of any record of this layer in the X-ray diagram and its failure to make any marked contribution to the optical properties of the wall in face view.

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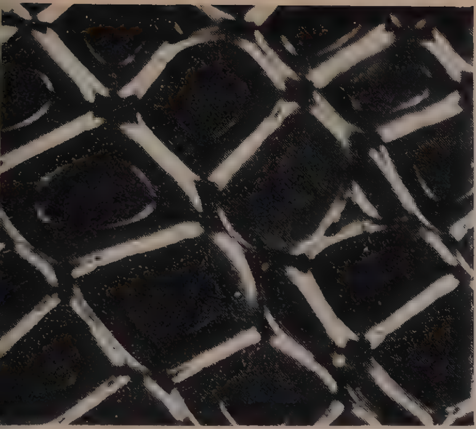

. 1. Thin (10 μ) transverse section of *Nothofagus cunninghamii* between crossed nichols.

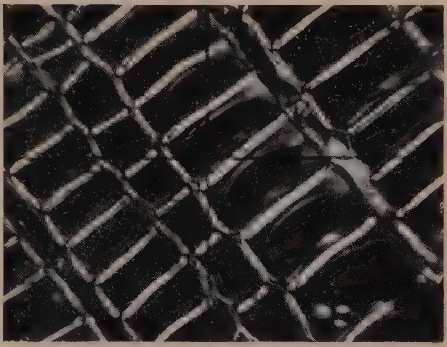

FIG. 2. Thin (10 μ) transverse section of *Picea* between crossed nichols.

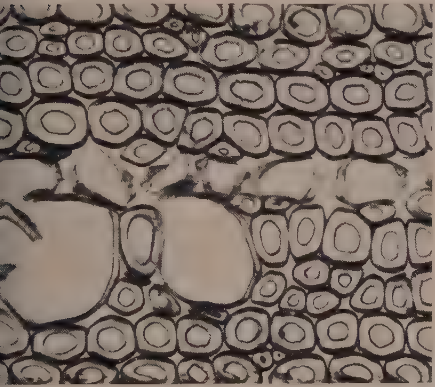

FIG. 3 *a*

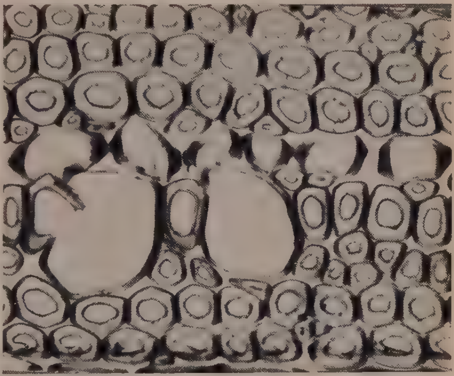

FIG. 3 *b*

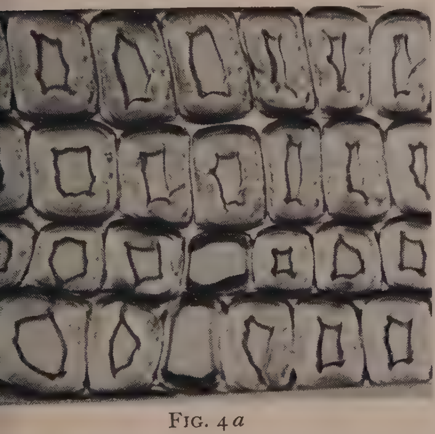

FIG. 4 *a*

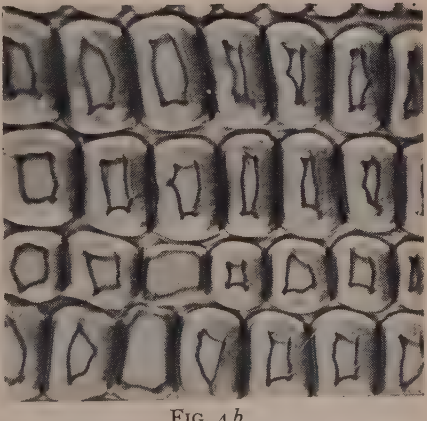

FIG. 4 *b*

FIGS. 3 and 4. Dichoism in transverse sections (15 μ) delignified and stained in Congo red.

IG. 3. *Nothofagus cunninghamii.* FIG. 4. *Picea.* In (*a*) the plane of vibration of the light is parrallel the short edge of the page. In (*b*) the plane of vibration of the light is perpendicular to the short edge of the page.

A. B. WARDROP and R. D. PRESTON.—PLATE I

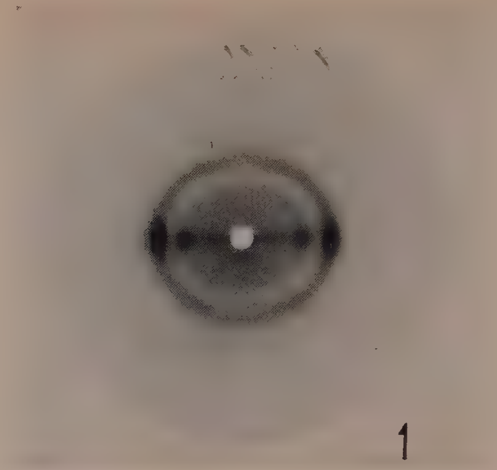

FIG. 1. X-ray diagram of wood of *Nothofagus cunninghamii* used in this investigation. Radiation Cu$K\alpha$, specimen-film distance 3 cm., beam perpendicular to grain, grain parallel to longer edge of pgea.

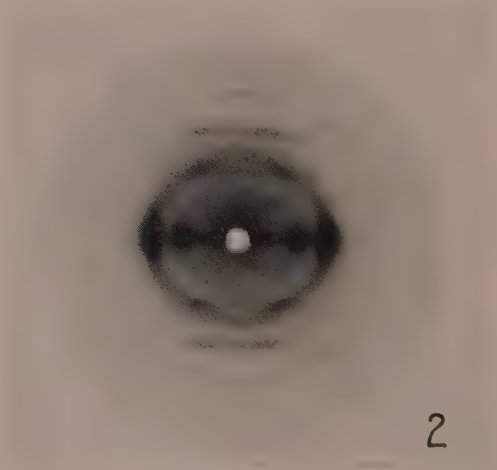

FIG. 2. X-ray diagram of wood of *Picea* sp. Details as in Fig. 1.

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PLATE II

Studies on the Germination of the Seeds of *Striga hermonthica*

II. THE EFFECT OF THE STIMULATING SOLUTION ON SEED RESPIRATION

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INTRODUCTION

It has been recently reported by Brown, Robinson, and Johnson (1949) that the natural stimulating solution which brings about the germination of the seeds of *Striga hermonthica* effects germination by promoting the extension of the cells of the radicle. These workers have also shown that cell-extension of pea-root segments takes place when they are treated with this same stimulating solution.

In view of the effect of auxin on cell-elongation and respiration (Pratt, 1938; Berger, Smith, and Avery, 1946; Kelly, 1947), it is to be expected that the respiration of the seeds of *Striga hermonthica* will be enhanced by the stimulating solution at the time when germination is taking place. This supposition is confirmed by Vallance (1949*b*), but, as is shown in this latter communication, the natural stimulating solution also enhances the respiration of the seeds of *Striga hermonthica* at times when they are unable to germinate.

The purpose of the present paper is to show, in more detail than previously reported, that whereas the stimulating solution is only able to bring about the germination of the seeds of *Striga hermonthica* at a definite stage in the complicated germination process, it is able to affect respiration at all stages of the germination cycle.

EXPERIMENTAL METHODS

The methods of preparing the stimulating solution, moisture-treating the *Striga* seeds, and inducing the germination of the *Striga* seeds were the same as those described in an earlier paper (Vallance, 1949*a*).

Initially, Dixon's modification of the Barcroft apparatus (Dixon, 1943) was used to measure respiration. This was found to be less satisfactory than the standard Barcroft, as it required 1 hour to reach equilibrium. After a few experiments, therefore, standard Barcroft respirometers were used instead of the modified respirometers. It was also noticed, in the early experiments when standard respirometer flasks were used, that some potassium hydroxide from the filter-paper roll always crept into the fluid at the bottom of the flasks. This contamination could be readily detected, for even 1 μl. of 20 per cent. potassium hydroxide in the 3 ml. of distilled water in the flasks markedly increased oxygen intake and also brought out the brown colour of the testas of the seeds into the surrounding water. Eventually this creeping was overcome by using flasks in which the centre cup was lipped like a test-tube. Except where otherwise stated, the temperature of the thermostatic bath was maintained at 32° C.

The respirometer flasks used with each respirometer were matched for volume as closely as possible. In consequence, it was considered that when the influence of the stimulating solution on seed respiration was being measured, possible effects of the stimulating solution on the composition of the gases inside the flasks could be neglected. When air-dry seed was added to flasks containing the stimulating solution, 40 mg. were used for each ml. of stimulating solution. When moisture-treated seed was employed care was taken to introduce approximately 70 mg. (dry weight) each time.

At the conclusion of each series of observations, the contents of each right-hand side respirometer flask were transferred to a small sintered crucible. The water was filtered from the crucible by means of a water-pump, and the seeds were dried for 15 hours at 80° C. and weighed. Results are expressed as μl. of gas at N.T.P. per mg. dry weight per hour. Six respirometers were used in all experiments. Thus when both oxygen consumption and carbon dioxide output were measured, three pairs of readings were obtained. As a result, in the figures illustrating these experiments each point plotted is the mean of three determinations. Each point plotted, in those figures which concern respiration in nitrogen, is the mean of six determinations.

Clearly it is desirable, when measuring respiration, to have full information about the respiratory substrate involved. In the work described in the present paper this was not possible, as sufficient material could not be obtained to enable full chemical analyses to be carried out. All that could be attempted was an initial determination of the gross reserves of the dry seed, and a study of the fate of the sugar of the seed during certain of the experimental periods. The precise identification of the carbohydrate of the dry seed was not possible, again due to lack of material, but qualitative analysis showed that the cell walls of the embryos were extremely thick, presumably acting as a reserve carbohydrate, that no starch was present, and that the seed contained a reducing sugar and a disaccharide.

The fate of these latter reserves at certain times was quantitatively determined by the method of Weinmann (1944, 1947). Results are expressed as mg. of hexose before hydrolysis and as mg. of increase in hexose content after hydrolysis.

Samples of *Striga* seed of three ages were used in the experiments. These were harvested in 1941, 1945, and 1948, respectively.

EXPERIMENTAL RESULTS

The effect of the natural stimulating solution on the dry seed of *Striga hermonthica* seemed likely to be of some value in determining its mode of action at other times. For, as reported previously (Vallance 1949*a*), after the dry seed of *Striga hermonthica* has been exposed to the natural stimulating solution at temperatures of either 22° C. or 32° C., its subsequent maximum germination capacity during a period of 21 days is of the order of 0·1 per cent. It seemed, therefore, that under these conditions the effect of the natural stimulating solution might be dissociated from its effect on germination and in consequence be more readily understood.

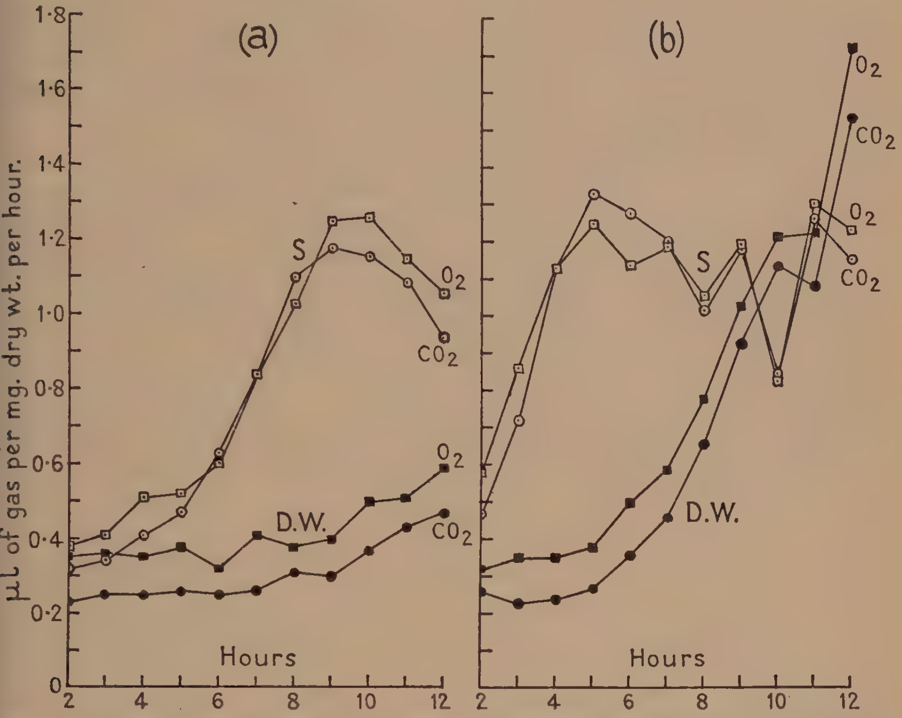

FIG. 1. The drift of respiration at 32° C. of air-dry seeds after being placed in the stimulating solution (S) and distilled water (D.W.). *a.* 1945 seeds. *b.* 1948 seeds.

With this aim in view, the drift of respiration was determined after air-dry seeds had been treated with the stimulating solution. Figs. 1*a* and 1*b* illustrate the respiration of the two samples of seed, one harvested in 1945 and the other in 1948, after being added to the stimulating solution. At the time of the respective experiments the 1945 seed was 2 years old and its maximum germination capacity was 50 per cent.; the 1948 seed was 4 months old and its maximum germination capacity was 20 per cent.

From Fig. 1*a* it may be seen that the enhancing effect of the natural stimulating solution on the respiration of the 1945 seeds compared with the controls was very considerable. At the time of peak respiration, during the 10th hour, oxygen intake was enhanced by 312·5 per cent., carbon dioxide output by 393·3 per cent. It will also be seen that the nature of the respiration of seeds treated with the stimulating solution was different from that of the controls. The nature of the former suggests that the substrate was carbohydrate; the nature of the latter, that the substrate used then was a fat, or alternatively protein plus a little carbohydrate. The identity of the substrate utilized when the stimulant is added to the dry seed is a matter of some importance, for, as demonstrated by Brown, Robinson, and Johnson (1949), it is highly probable that the active principle of the stimulating solution is similar to D-xylo-ketose. Thus it might be argued that this sugar was utilized as the substrate for the augmented respiration. As far as the 1945 seed was concerned sufficient material was not available for analysis, so that from the data obtained using this seed it is impossible to decide whether or not this supposition is correct. With regard to the 1948 seed chemical analysis showed that the sugar of the seeds decreased during the experimental period.

The data shown in Fig. 1*b* are somewhat different from those of Fig. 1*a*. It will be seen from this figure that the stimulating solution enhanced the respiratory intensity, compared with the control, up to the 9th hour. After the 11th hour the rate of the gaseous exchange in the control was higher than that of the seeds in contact with the stimulating solution. This latter figure suggests that although the stimulating solution enhances the respiratory process, it does not, as might appear from Fig. 1*a*, act as a respiratory substrate. The data shown in Table I confirm this view, for the sugar losses shown would provide sufficient substrate for the respiration in both the distilled water and stimulant-treated seeds during the 12-hour experimental period.

TABLE I

	Sugar content in mg. per gm. dry wt.	
Nature of treatment	Reducing sugar	Increase in reducing sugar after hydrolysis
Air-dry seed	14·0	14·7
	12·5	13·2
12 hours at 32° C. in distilled water	1·8	10·2
	2·4	12·4
12 hours at 32° C. in the stimulating solution	1·0	7·7
	1·2	6·6

The stimulating solution also enhances respiration at 22° C. This may be seen from Fig. 2. After 12 hours oxygen uptake by seeds treated with the stimulating solution was 250 per cent. greater than that of seeds exposed to distilled water. Comparison of this figure with Fig. 1*b* shows that at the 9th hour the Q10 for the effect of the stimulant on respiration was approximately 2. This suggests that the active principle of the stimulating solution has an effect on some link of the respiratory chain.

The data presented in Figs. 1 and 2 show that the natural stimulating solution is capable of enhancing the respiration of the seeds of *Striga hermonthica* at a time when they are unable to germinate. These results, however, give no indication of the mode of action of the stimulant. As a further step in the elucidation of this problem, the effect of the natural stimulating solution on the respiration of the seeds in nitrogen was investigated. It was found in all experiments of this type that the stimulating solution enhances carbon-dioxide output compared with the controls.

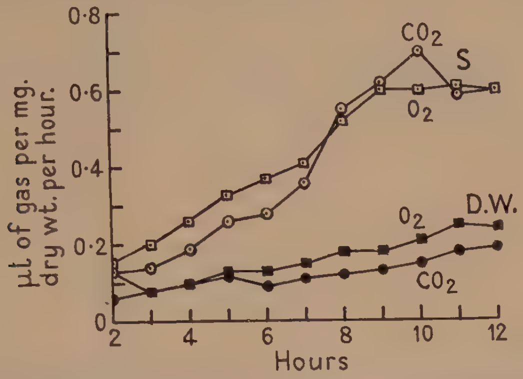

FIG. 2. Respiration of air-dry 1948 seeds at 22° C. after being added to the stimulating solution (S) and distilled water (D.W.).

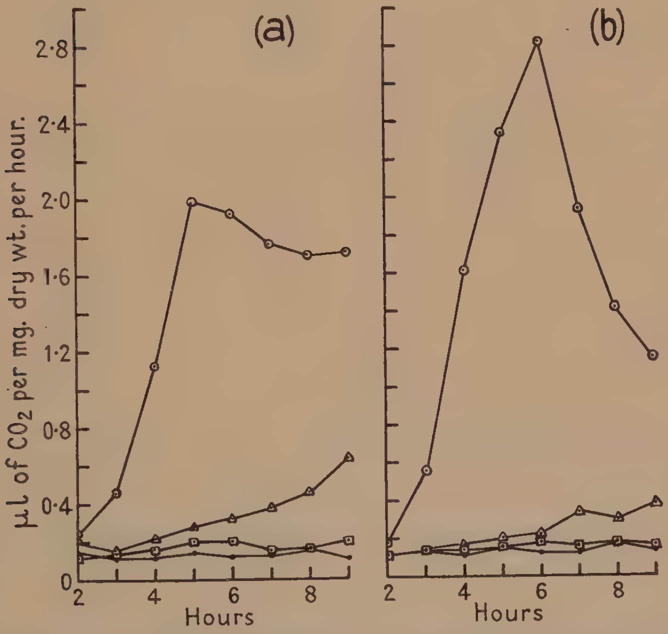

FIG. 3. The drift of CO_2 output in nitrogen at 32° C. Stimulant solution (–⊙–), distilled water (–⊡–), stimulant+0·025 M. NaF (–△–), stimulant+0·001 M. Iodoacetate (–•–).

Figs. 3*a* and 3*b* illustrate the results obtained at a temperature of 32° C. when air-dry seeds were added to the stimulating solution and to distilled water, and were then allowed to respire in nitrogen. The data relate to 1941 seeds. It will be seen that the enhancing effect of the stimulant on CO_2 output is much greater in nitrogen than in air. The addition of sodium fluoride and sodium monoiodoacetate to the stimulating solution, to give 0·025 M.

and 0·001 M. solutions of these poisons respectively, further showed that the respiratory system involved in the increase is sensitive to these poisons.

It is clear, therefore, that the stimulating solution is capable of enhancing the aerobic and anaerobic respiration of *Striga* seeds during treatment which does not allow germination to take place. The effect of the stimulating solution on respiration is still marked when seeds are treated with it after prior exposure to moisture for various periods. Enhanced respiration takes place, after seeds are treated with the stimulating solution, even though the requisite conditions for germination have not been established inside the seeds. This may be seen from Fig. 4*a* and 4*b*. Fig. 4*a* relates to 1948 seed, Fig. 4*b* to 1945 seed. Each sample of seed had been moistened with distilled water and maintained at 22° C. for 6 days before being exposed to the stimulating solution. Concurrent experiments showed that, at the time of the observations illustrated by the figures, the germination capacity of the 1948 seed was zero, that of the 1945 seed 50 per cent.

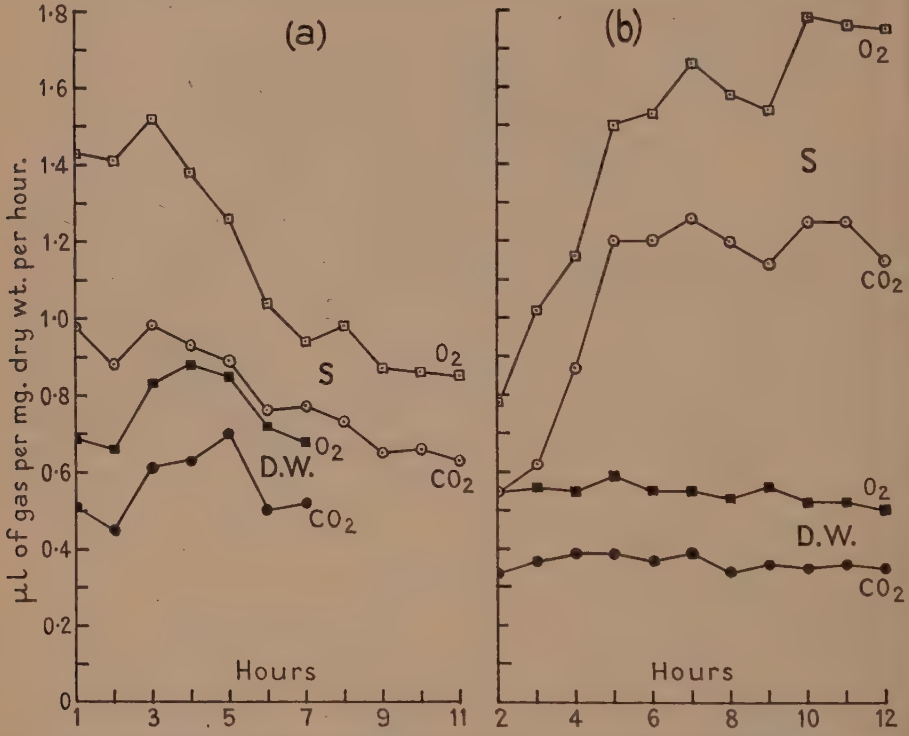

FIG. 4. Respiration of moisture-treated seeds at 32° C., in contact with the stimulating solution (S) and distilled water (D.W.). *a*. 1948 seeds. *b*. 1945 seeds.

The data given in Fig. 4*a* show that the respiration in the presence of the stimulating solution was higher than in the control experiment, but that as the experiment proceeded the intensity fell markedly. Fig. 4*b* shows that when the seeds are able to germinate, as a result of prior moisture-treatment, the enhanced respiration compared with the control does not diminish after 12 hours.

From both Fig. 4*a* and 4*b* it will be observed that the nature of the respiration, resulting from the treatment of the pretreated seeds with the stimulating solution, is different from that shown in Fig. 1*a* and 1*b*. Analysis of a sample of 1941 seed, after the same treatment, showed that during the experimental period of 12 hours the sugar content did not vary and that the weight of the crude ether extract was the same at the beginning and end of the experimental period. It was therefore concluded that the stimulating solution is capable of enhancing a protein respiration and that the respiration due to the stimulant, shown in Fig. 4*a* and 4*b*, was essentially protein respiration.

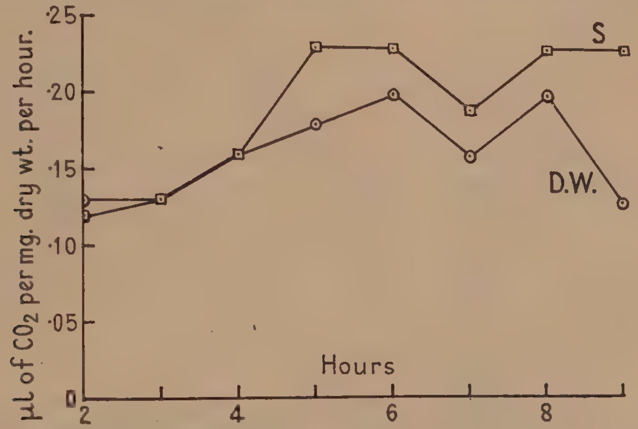

FIG. 5. Respiration in nitrogen at 32° C. after moisture-treatment, in contact with the stimulating solution (S) and distilled water (D.W.). (Standard error of each plotted mean = ±0·02.)

A further point of interest was noted when 1945 seeds were allowed to respire in nitrogen after prior aerobic moisture-treatment for 6 days at 22° C. At this time, as shown by Fig. 5, it was found that the stimulant had little significant effect on the anaerobic output of carbon dioxide compared with the anaerobic carbon dioxide output of seeds maintained in distilled water. This result, while differing from those illustrated in Figs. 3*a* and 3*b*, was more in accordance with expectation, for it was found that anaerobic conditions inhibit *Striga* germination. It will be noticed that in this latter respect there is some similarity between *Striga* germination and the extension growth of *Avena* coleoptiles, which, as shown by Bonner (1933), will not elongate in an atmosphere of nitrogen.

Later the stimulating solution was found to enhance the aerobic respiration of seeds after they had been exposed to moisture for various periods at various temperatures. It was found to enhance the respiration of 1945 seeds throughout the whole of a pretreatment cycle during which the germination capacity of the seed varied, as shown in an earlier paper (Vallance, 1949*a*) from 0 per cent. to 50 per cent. In this experiment it was found that after 33 days of pretreatment the germination capacity of the seed was only 7 per cent. Nevertheless the stimulant still markedly affected the respiration of the seeds. This may be seen from Fig. 6. Comparison of this figure with Fig. 1*a* and Fig. 4*b* shows that the maximum carbon-dioxide output was much the same in all three cases. It will also be seen that the oxygen consumption resulting from the treatment of the seeds with the stimulating solution is similar in Fig. 1*a* and Fig. 6.

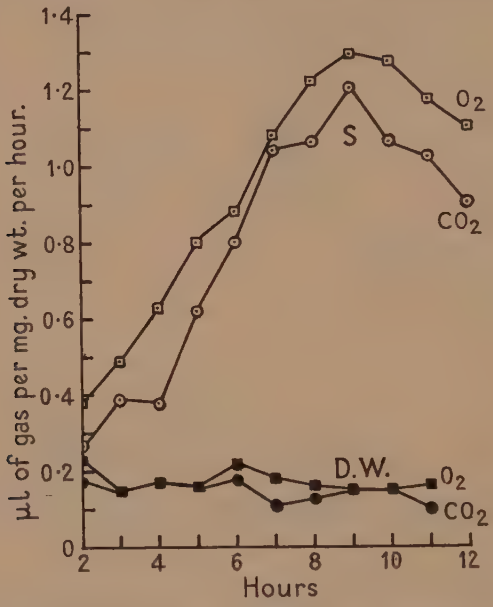

FIG. 6. Respiration of 1945 seeds at 32° C. after moisture-treatment for 33 days at 22° C. in stimulating solution (S) and distilled water (D.W.).

From the data of the experiments mentioned above and others, it was not possible to show any correlation between the germination capacity of the seeds at any time and the concomitant respiration due to the stimulant. Yet in spite of this it seemed possible that at any one time some such correlation might exist. To test this possibility samples of a batch of 1941 seed were aerobically moisture-treated for 6 days at 22° C. and were then exposed to various dilutions of the stimulating solution. The stimulating solution was diluted with distilled water. The respiration and the percentage germination induced by each concentration of the stimulating solution were determined at the same time. A representative set of data are shown in Fig. 7, and from this figure it will be seen that there was no correlation between germination and carbon-dioxide output. The data for oxygen consumption showed no correlation with germination either.

It may be argued that, as the maximum germination percentage induced by treating the 1941 seed with the undiluted stimulating solution was only 20·6 per cent., the results are less valid. Nevertheless the results are in accord with other experimental evidence and thus it is considered that they may be provisionally accepted. However, when the requisite amount of a more freely germinable sample of seed of *Striga hermonthica* becomes available, further experiments on the effect of diluting the stimulating solution will be carried out.

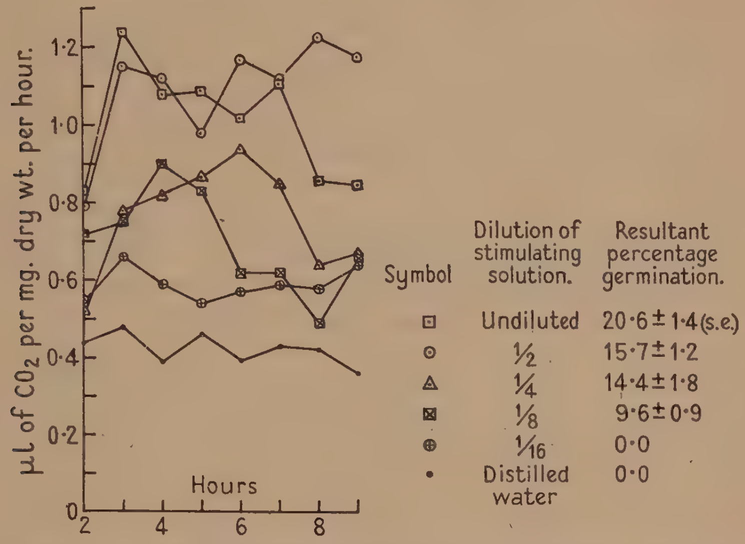

FIG. 7. The effect of diluting the stimulating solution on respiration and germination.

DISCUSSION

The data presented show that a stimulating solution, prepared by allowing the roots of seedlings of a variety of *Sorghum vulgare* to grow in distilled water, is capable of raising the level of respiration of the seeds of the hemi-parasite *Striga hermonthica*. The respiration of the seeds treated with this solution, compared with that of seeds moistened with distilled water, is enhanced even though the seeds are unable to germinate. Clearly, it is to be expected that some increase in respiration will be necessary at the times when germination is taking place, but the significance of the increase, at times when the seeds are unable to germinate, is obscure.

If the effect of the stimulating solution on germinable seeds is primarily to increase respiration, then it may be, as suggested by Brown and Robinson (1949), that this in turn promotes cell extension in the radicle. In this case it might be expected that some correlation would exist between respiration and germination, but so far it has not been possible to establish this correlation. Furthermore, if the only effect of the stimulating solution is on respiration, then it would be expected that traces of potassium hydroxide would also promote germination at the appropriate stage in the germination process, since it is known to affect markedly the respiration of seeds at this stage. Such an effect on germination of *Striga hermonthica* seeds has not been demonstrated.

It would seem, therefore, that the effects of the stimulating solution on respiration and cell elongation may, at least in part, be independent.

SUMMARY

1. Data are presented which show the effect of the natural stimulating solution on the respiration of the seeds of *Striga hermonthica*.

2. The stimulating solution has been shown to enhance the aerobic respiration of seeds exposed to it, compared with that of seeds to distilled water at the same temperature. This effect on the respiration of the seeds is quite independent of any previous moisture-treatment of the seeds, and thus of germination.

3. The maximum anaerobic rate of carbon-dioxide output of air-dry seeds after treatment with the stimulating solution may be up to fifteen times greater than that of seeds moistened with distilled water. The anaerobic respiration of air-dry seeds after exposure to the stimulating solution is greatly reduced by 0·025 M. sodium fluoride and 0·001 M. sodium monoiodoacetate.

4. The stimulating solution has been found to have little effect on the anaerobic output of carbon dioxide from seeds which had been moisture-treated for 6 days at 22° C. before treatment with the stimulating solution.

5. No correlation could be established between the effects of the stimulating solution on germination and respiration.

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A Comparison of the Aerobic and Anaerobic Respiration of Apples

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SUMMARY

1. The loss of carbohydrate, acid, and cell-wall material, and the production of carbon dioxide and alcohol, have been studied in apples under aerobic and anaerobic conditions.

2. The changes in all these metabolites, except the cell-wall material, proceed in a regular manner.

3. The presence of oxygen has a conserving effect on the loss of carbohydrate.

4. The presence or absence of oxygen is without effect on the rate of loss of acid.

5. The rate of loss of acid is proportional to the logarithm of its concentration and is constant for a given variety of apple.

6. The loss of carbohydrate plus acid accounts quantitatively for the production of carbon dioxide and alcohol, both in air or in nitrogen.

7. The anaerobic respiration of carbohydrate by apples is identical, so far as the nature and quantity of the end products are concerned, with the alcohol fermentation of yeast.

8. The carbon dioxide which is produced in nitrogen, over and above that produced in fermentation, is equivalent to that which would be produced by oxidation of the acid.

INTRODUCTION

MANY tissues of the higher plants accumulate ethyl alcohol in the absence of oxygen. Although the formation of alcohol is usually represented by the equation: $C_6H_{12}O_6 \rightarrow 2CO_2+2C_2H_5OH$, which would give a value of 1·0 for the quotient (in molecular equivalents) of carbon dioxide/alcohol, it has long been known that this quotient generally exceeds unity. For example, Kostytschew (1913) and Boysen-Jensen (1922), working with flowers, seeds, roots, tubers, leaves, and fruits, record the theoretical value in two instances only, namely, with flowers of *Acer platinoides* and roots of *Daucus carota*. Using apples, Kostytschew records 1·25 for the variety 'Sinap' and 2·5 for 'Anton', and Fidler (1933) found values ranging from 1·05 to 1·8 for 'Bramley's Seedling' and 'Newton Wonder' apples in several seasons.

It has been variously suggested (e.g. see Kostytschew, 1927) (*a*) that alcohol is formed and is afterwards converted into other substances, (*b*) that part of the substrate is converted into some compound which escapes estimation, and (*c*) that the excess of carbon dioxide arises from some process not connected with the catabolism of carbohydrate. There has, however, been no previous investigation of the problem.

In the investigation described in this paper the production of carbon dioxide and alcohol by apples, respiring in air and in the absence of oxygen, were determined, as were the changes in the concentrations of carbohydrates, acids, and the alcohol-insoluble residue (i.e. cell-wall material). Using mature apples harvested at the normal time, it has been shown that the loss of carbon as carbohydrate plus acid was equal to the production of carbon as carbon dioxide plus alcohol, in air and in nitrogen; that the rate of loss of acid was unaffected by the presence or absence of oxygen; and that under anaerobic conditions the amount of carbon dioxide plus alcohol which was produced was equivalent to the sum of the carbon dioxide and alcohol which would have resulted from fermentation of the carbohydrate lost, plus the amount of carbon dioxide which could be produced from complete oxidation of the acid lost. In apples the relative rates of loss of carbohydrate and acid determine the magnitude of the quotient carbon dioxide/alcohol; this quotient (in molecular equivalents) tends to unity as the loss of acid tends to zero.

MATERIALS

Two varieties of apple have been used, namely, Sturmer Pippins grown at the East Malling Research Station, and Bramley's Seedlings grown at Cottenham, Cambridge, or Patrixbourne, Canterbury. During the years 1933 to 1938 several bushels of these varieties were harvested at the normal date (from 22 September to 15 October) and sent to the laboratory, where they were stored at a constant temperature of 3° C. The apples were green when received, but they slowly changed colour to yellow in storage. When used for experiments early after harvesting they contained starch, which disappeared as the experiments progressed, but later in the storage season they were starch-free before the experiments began.

METHODS

1. *Experimental*

Before each experiment a sufficient number of apples was taken from the stock in cold-storage to form the number of samples needed. Each sample was usually of the order of 15 to 20 fruits, of total weight from 1·2 to 2·2 kg., but occasionally the samples consisted of as many as 40 fruits. If it was decided to analyse the apples after, say, five successive periods of time, then 11 to 13 samples would be selected, 5 for the series in air and 5 in nitrogen, with 1 to 3 'initial' samples.

The apples were weighed, marked on the skin with the number of the sample in indian ink, and placed in large cylindrical glass jars. The jars were sealed with glass disks smeared with luting wax, carrying inlet and outlet tubes for ventilation. These containers were then placed in a constant-temperature room at 3°, 12°, or 20° C., and ventilated with fresh air (from which carbon dioxide had been removed) drawn from outside the building, at a rate which was sufficient to keep the concentration of carbon dioxide at about 0·5 per cent.

After 4 or 5 days the samples which were to be used to give an estimate of the initial concentrations of carbohydrates, acid, and alcohol were weighed and frozen in biscuit tins in a room at −20° C. At the same time measurements of the production of carbon dioxide by the remaining apples were begun, and nitrogen was substituted for air in the anaerobic samples.

In two of the experiments, namely, Nos. 1 and 141, the nitrogen was used direct from cylinders and contained from 0·2 to 0·6 per cent. of oxygen. In all other experiments the nitrogen was freed from oxygen by passage over copper gauze heated to dull red heat and, in a few experiments, by subsequent passage through alkaline pyrogallol in a bead tower. It was never possible to detect oxygen in the purified nitrogen by analysis in a Haldane apparatus.

From the jars containing the samples used to determine the rate of respiration, the stream of air or nitrogen bubbled through concentrated sulphuric acid, to trap transpired alcohol (Fidler, 1934), and then through potassium hydroxide solution in a Pettenkofer tube. These tubes were changed when the potash had absorbed about one-third of its maximum capacity of carbon dioxide. After intervals of time which were either decided before the experiment began, or which depended on the rate of production of ethyl alcohol by the anaerobic fruit, samples were removed from air and nitrogen and frozen at −20° C. At the same time the acid in the trap bottles was renewed.

2. *Analytical*

i. *Carbon dioxide* was determined by absorption in caustic potash solution, ranging from 0·1 N to 2 N (depending on the rate of production), and titrated with 0·1 N to N hydrochloric acid by the method normally used for determining carbonate in hydroxides, i.e. first to a phenolphthalcin and then to a methyl orange end-point.

ii. *Ethyl alcohol and acetaldehyde.* These substances were estimated by methods previously described (Fidler, 1934). Because the bulk of the samples had to be preserved in a frozen state for carbohydrate analyses, only part of the fruit could be distilled for the zymasis estimates. In practice, longitudinal radial segments were cut from each apple, removing one-eighth to one-quarter of the tissue, for distillation. The remainder was frozen at −20° C.

The values given in Table I include not only the alcohol accumulating in the fruit but also the transpired alcohol, i.e. that trapped in the sulphuric acid. The quantity transpired amounted to from 2 to 15 per cent. of the total, depending on the variety of apple, the temperature, and the concentration of alcohol in the tissues; in other words, it was not negligible.

iii. *Carbohydrates and acid.* These were estimated by the methods[1] evolved by Archbold and her colleagues, of the Imperial College, London, except that hydrolysis of sucrose was effected by 0·6 N hydrochloric acid, for 25 minutes at 60° C., instead of using citric acid. In Expt. 1 starch was estimated by the method of Bish (1929), using taka-diastase, but in all subsequent experiments Hanes's (1936) β-amylase method was used.

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[1] A summary of these methods is given by Onslow, Kidd, and West (1931). The original papers include those of Lane and Eynon (1923), Archbold (1928), and Evans (1928).

TABLE I

Loss of carbon as carbohydrate, acid, and alcohol-insoluble residue, and accumulation of carbon as carbon dioxide and ethyl alcohol. (See 'Notes to Table I', p. 46)

				Milligrams carbon per 100 grammes fresh weight of apple					Ratio of molecules carbon dioxide to molecules ethyl alcohol
						Loss, or gain (+) of			
Number of experiment and sample	Date begun	Conditions	Days in gas	Production of carbon dioxide	Increase in alcohol number	Carbohydrate	Malic acid	Alcohol insoluble residue	
Column 1	2	3	4	5	6	7	8	9	10
A. *Sturmer Pippin apples*									
1 ABC	22.10.33	Air. 20° C.	0						
L			2	23	0	144	31	—	—
M			4	47	0	+44	33	—	—
N			8	105	0	+12	46	165	—
O			12	160	0	48	66	—	—
P			20	282	0	92	112	125	—
Q			30	404	0	280	141	215	—
R			45	560	5	448	191	185	—
1 ABC	21.10.33	0·2 to 0·6 % oxygen, rest nitrogen 20° C.	0						
D			2	33	28	60	17	—	100:42
E			4	59	40	92	14	—	100:34
F			8	90	67	292	42	140	100:37
G			12	125	101	144	80	—	100:41
H			20	200	151	196	105	140	100:38
I			30	287	219	416	152	135	100:38
J			45	410	355	720	189	155	100:43
55 E	4.12.34	Air. 3° C.	0						
D			31	147	1	+76	32	40	—
C			44	212	2	256	79	+80	—
B			64	317	2	180	82	+60	—
A			112	573	9	352	125	+10	—
53 E	4.12.34	Pure nitrogen. 3° C.	0						
D			31	126	130	8	4	+15	100:52
C			44	169	177	292	61	105	100:52
B			64	235	167	428	86	130	100:36
A			112	377	297	584	125	110	100:40
58 C	30.11.34	Air. 12° C.	0						
B			20	257	2	292	86	+85	—
A			39	486	1	264	129	+110	—
DE			53	658	0	56	133	70	—
56 C	30.11.34	Pure nitrogen. 12° C.	0						
B			20	175	213	432	93	+30	100:61
A			39	327	432	716	147	65	100:66
DE			53	432	468	736	175	35	100:55
61 B	28.11.34	Air. 20° C.	0						
A			9	213	0	+48	36	+50	—
C			21	475	5	56	107	145	—
DE			35	781	2	532	157	85	—
59 C	28.11.34	Pure nitrogen. 20° C.	0						
B			9	164	193	152	39	+115	100:70
A			21	339	494	668	106	90	100:73
DE			35	543	850	1380	172	15	100:78

TABLE I (*continued*)

Number of experiment and sample	Date begun	Conditions	Days in gas	Milligrams carbon per 100 grammes fresh weight of apple					Ratio of molecules carbon dioxide to molecules ethyl alcohol
				Production of carbon dioxide	Increase in alcohol number	Loss, or gain (+) of			
						Carbohydrate	Malic acid	Alcohol insoluble residue	
Column 1	2	3	4	5	6	7	8	9	10
A. *Sturmer Pippin apples (continued)*									
62 AB	7.12.34	Air. 12° C.	0						
64			12	186	0	148	43	35	—
66			62	841	4	652	179	235	—
67 A			27	415	0	216	100	+50	—
62 AB	7.12.34	Pure nitrogen. 12° C.	0						
65			15	156	260	380	64	25	100:84
71 AB	1.2.35	Air. 12° C.	0						
73			13	161	1	+120	57	15	—
71 AB	1.2.35	Pure nitrogen. 12° C.	0						
74			13	161	209	320	79	20	100:65
76 AB	28.2.35	Air. 12° C.	0						
77			34	440	2	452	107	80	—
138 AC	5.12.36	Air. 12° C.	0						
139 C			16	169	0	132	52	40	—
B			38	389	3	378	122	17	—
A			56	540	4	878	141	5	—
138 AC	5.12.36	Pure nitrogen. 12° C.	0						
140 C			16	155	187	188	45	40	100:61
B			38	301	380	506	105	25	100:63
A			56	421	538	814	124	+4	100:64
138 AC	5.12.36	0·6 % O_2 99·4 % N_2 12° C.	0						
141 C			16	124	139	140	43	35	100:57
B			38	254	272	504	99	20	100:54
A			56	366	418	708	125	0	100:57
143 AC	14.12.36	Air. 12° C.	0						
144 A			10	115	0	229	44	34	—
B			20	239	3	163	65	46	—
C			30	345	2	348	93	50	—
D			40	447	5	338	118	2	—
E			50	549	5	392	152	59	—
F			60	644	11	462	162	+18	—
G			70	735	6	564	189	49	—
143 AC	14.12.36	Pure nitrogen. 12° C.	0						
145 A			10	115	109	407	27	16	100:48
B			20	211	245	514	80	12	100:59
C			30	294	364	761	99	+31	100:62
D			40	364	495	869	118	28	100:68
E			50	443	645	1120	140	+9	100:72
F			60	529	771	1164	167	0	100:73
G			70	613	871	1356	192	27	100:71
B. *Bramley's Seedling apples*									
79 AB	28.2.35	Air. 12° C.	0						
80 B			4	46	5	+100	18	20	—
C			8	90	10	248	48	55	—
D			12	133	10	32	52	35	—
A			20	215	15	244	61	+5	—
83			33	329	38	236	115	20	—

TABLE I (*continued*)

Number of experiment and sample	Date begun	Conditions	Days in gas	Milligrams carbon per 100 grammes fresh weight of apple					Ratio of molecules carbon dioxide to molecules ethyl alcohol
				Production of carbon dioxide	Increase in alcohol number	Loss, or gain (+) of			
						Carbohydrate	Malic acid	Alcohol insoluble residue	
Column 1	2	3	4	5	6	7	8	9	10
B. *Bramley's Seedling apples (continued)*									
79 AB	28.2.35	Pure nitrogen. 12° C.	0						
82 B			4	46	73	76	29	+5	100:78
C			8	85	120	+12	39	+65	100:71
D			12	115	146	284	47	80	100:63
A			20	172	245	248	64	40	100:70
81			20	153	213	476	79	80	100:70
96 AE	2.11.35	Air. 12° C.	0						
97 A			2	19	7	+72	0	+15	—
B			4	39	0	+36	+14	20	—
C			6	58	3	60	0	+35	—
D			9	87	5	+32	7	35	—
E			12	114	7	56	14	0	—
F			16	148	11	112	39	50	—
G			20	181	12	68	50	30	—
H			30	258	15	200	65	35	—
I			40	333	27	452	86	45	—
96 AE	2.11.35	Pure nitrogen. 12° C.	0						
98 A			2	22	37	+100	50	5	100:83
B			4	43	58	108	14	+5	100:67
C			6	61	87	40	21	5	100:71
D			9	89	100	+12	43	+5	100:57
E			12	115	130	120	29	+25	100:57
F			16	143	177	340	82	0	100:62
G			20	166	184	152	50	+35	100:56
H			30	219	220	324	111	20	100:48
I			40	264	269	492	129	15	100:51
99			20	128	165	396	79	20	100:65
158 C	17.11.38	Air. 12·7° C.	0						
158 A			26	183	23	116	36	—	—
159 C	26.1.39	Air. 12·7° C.	0						
159 E			25	179	19	132	29	—	—

Notes to Table I.

Column 2: Date of freezing of initial samples.

4: Days from date of freezing of initial samples (initials indicated by '0').

6: i.e. ethyl alcohol+acetaldehyde.

9: Total residue minus starch.

Experiments 55 to 59: The initial samples were lost. The first samples analysed on removal from gas were used as initials for subsequent samples. Thus in 53, 56, 59, respectively, the apples had been in nitrogen for 14, 10, and 8 days longer than the times given in column 4.

The estimation of malic acid was by titration, the acidity being calculated as 'mg. malic acid'. This assumes (*a*) that no other acids are present, and (*b*) that any malate present does not liberate malic acid in storage. There are good grounds for believing that both assumptions are correct. The evidence for (*a*) will be presented in a later paper on acid metabolism in gas-storage of apples; Kidd and Hanes (1936) showed that the drift in pH of the juice of apples in storage is caused by a decreasing concentration of free acid in the presence of a constant, small, amount of monobasic salt. Thus differences between titratable acidity in consecutive samples are true reflections of changes in total malic avid.

iv. *Standard errors of the above estimates.* Because the construction of a progress curve for change of concentration of alcohol, carbohydrate, or acid involves the analysis of a separate sample of apples for each point, serious errors are introduced by sampling. Experiments were carried out to determine the standard deviations of the analytical data, with the following results. The values are expressed as milligrams of carbon per 100 grammes fresh weight of fruit, and refer to samples of from 15 to 20 fruits each.

Total sugar, $\sigma = 64$ (calculated from 14 observations).
Alcohol insoluble residue, $\sigma = 20$ (calculated from 12 observations).
Acid, $\sigma = 18$ (calculated from 14 observations).
Carbon dioxide, $\sigma = 1{\cdot}9$ per cent. of the value of the estimate.
Ethyl alcohol plus acetaldehyde (i.e. 'the alcohol number'), $\sigma = 2{\cdot}3$ per cent. of the value of the estimate.

RESULTS

The primary data for the loss of carbohydrate and acid, for the accumulation of alcohol, and for the production of carbon dioxide, are given in Table I. To facilitate the subsequent analyses of these data all values are expressed in terms of milligrams of carbon per 100 grammes initial fresh weight of apple. Table I also gives details of the experimental conditions. In those experiments where the apples initially contained starch the loss of starch has been included in the figure for carbohydrate.

Data are available for the change of concentration of sucrose, glucose, and fructose in Expts. 1, 97–8, and 144–5, but they have not been included. This is partly because the graphs for the drift in concentration of total glucose and total fructose are dissimilar in the three experiments, and also because of the existence of more extensive data, to be published elsewhere by Kidd and West.

DISCUSSION

The results are discussed in two main sections. In the first the changes in concentration of the substances estimated are treated in general terms, since much of what is recorded here has been noted earlier by other workers. Thus the rate of production of carbon dioxide by apples in air and in nitrogen is discussed by Blackman and Parija (1928) and Parija (1928). Thomas (1925) studied the rate of production of ethyl alcohol by apples in nitrogen, and Kidd and West[1] have published data on changes of concentration of carbohydrates and acid in apples stored in air. What is new in the present work is that these estimates have been obtained simultaneously on comparable samples of apples, stored both in air and in nitrogen. Further, the periods of anaerobiosis were, with the exception of those in Thomas's experiments, considerably longer than in experiments previously recorded in the literature.

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[1] In numerous reports in the Annual Reports of the Food Investigation Board, 1930 to 1939.

In the second section the data are analysed statistically.

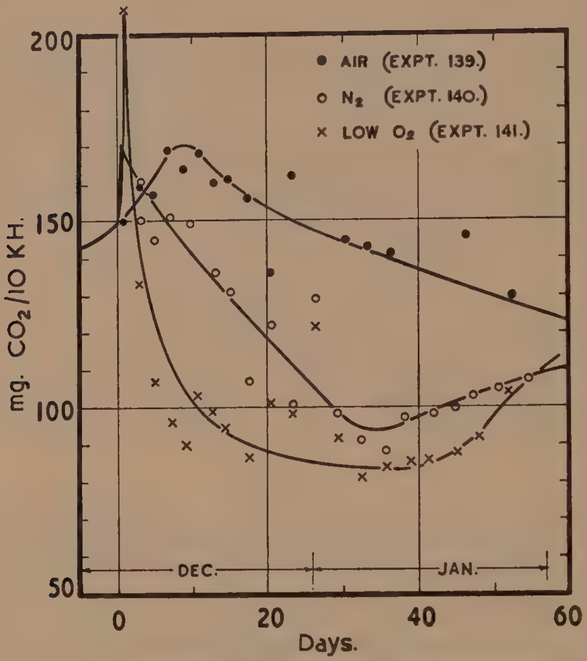

FIG. 1 *a*. Respiration of pre-climacteric Sturmer Pippins, 1936–7, at 12° C.

1. *General trends of the metabolites*

Certain of the experiments, which gave typical results, have been selected for discussion. These are the Expts. 143–5, using Sturmer Pippins, and 97–8, on Bramley's Seedlings. They were both experiments of long duration, in which samples were analysed frequently. However, Expts. 138–41 have been used instead of Expts. 143–5 to illustrate rates of production of carbon dioxide by Sturmer Pippins, because this series included samples respiring in nitrogen containing a low concentration of oxygen.

i. *Carbon dioxide.* Graphs of the rate of production of carbon dioxide are given in Figs. 1, *a* and *b*. Fig. 1 *a* (Sturmer Pippin apples at 12° C., 1936) illustrates the behaviour of apples which had not undergone the climacteric of respiration when the experiments began. The curve for rate of respiration in air is typical of pre-climacteric apples. When the supply of oxygen is removed there is a transitory slight stimulation of the rate of output of carbon dioxide, but the main effect is a marked fall in the rate. This continues for a long time (more than 30 days at 12° C. in 1936 and 20 days at 20° C. in

Expt. 1, 1933), after which the rate of production of carbon dioxide increases. This may correspond to the climacteric, reached much later than in air. The graph for Expt. 141 on Fig. 1 *a* illustrates the effect of about 0·5 per cent. of oxygen in the stream of nitrogen; the form of the curve is similar to that for production of carbon dioxide in pure nitrogen, but the general level s lower, considerably so for the first 20 days.

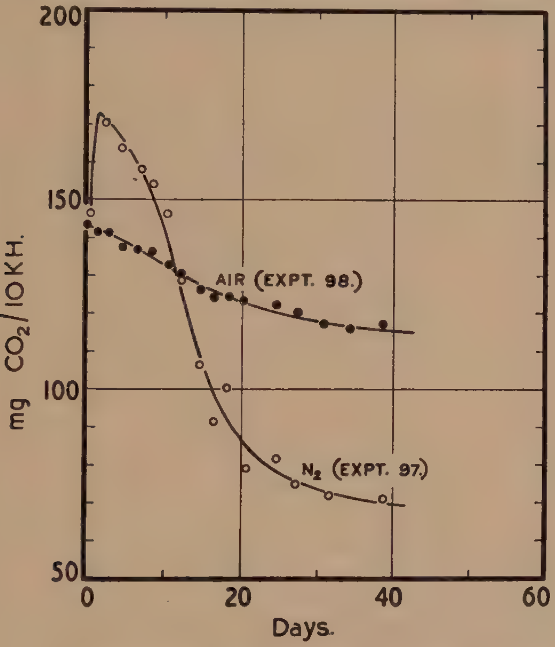

FIG. 1 *b*. Respiration of post-climacteric Bramley's Seedlings, 1935–6, at 12° C.

There was no experiment on post-climacteric apples which lasted long enough to show whether there was an increase in respiration after very long exposure to nitrogen, but Fig. 1 *b* is typical of post-climacteric apples. The air line drifts regularly downwards, but the nitrogen line shows a rate of production of carbon dioxide greater than that in air, for a long time (about 11 days in Expts. 97–8, on Bramley's Seedlings at 12° C., in 1935). The subsequent drift below the air line is checked by day 30, but there is no sign of any rise by day 40.

ii. *Ethyl alcohol.* The graphs of Fig. 2 actually illustrate the increase in the alcohol number, which includes acetaldehyde, but since in nitrogen acetaldehyde forms 1 per cent. or less of this number, they can be used to show the trend for alcohol alone.

Sturmer Pippin apples, particularly when pre-climacteric, withstand anaerobic conditions very well, and usually show no injury even after very long periods of exposure. In Fig. 2 it can be seen that the production of ethyl alcohol was being maintained at a high rate after 70 days (12° C., 1936, Expt. 145), when the concentration of ethyl alcohol had reached 1·6 per cent. in the tissues.

Bramley's Seedling apples (Expt. 98, 1935, 12° C.) do not produce alcohol as rapidly as Sturmer Pippins, and the slope of the graph for concentration of alcohol declines regularly during anaerobiosis. This variety shows some injury when the concentration of ethyl alcohol reaches about 0·7 to 1 per cent.

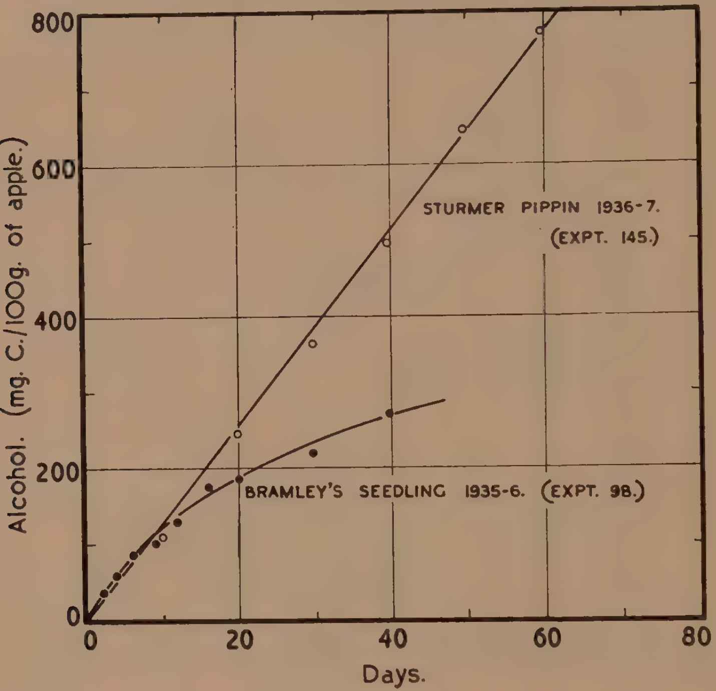

FIG. 2. Accumulation of alcohol in apples in nitrogen at 12° C.

The figures given in column 10 of Table I illustrate the range of values of the ratio $CO_2 : C_2H_5OH$ in the anaerobic respiration of apples. As previously noted, the ratio always shows an excess of carbon dioxide.

iii. *Carbohydrates.* The estimates for carbohydrate content of the apples are subject to greater errors than the other estimates discussed in this paper, and the initial sample (from which all changes in concentration are calculated) is subject to the same errors as subsequent samples. Thus the graphs in Figs. 3 *a* and *b* (which show the change in concentration of carbohydrates) differ from those of Fig. 2, because the line need not pass through the zero value. The cross-hatched area on the graphs extends from 2σ above to 2σ below the initial value. In either Figs. 3 *a* or 3 *b* lines drawn by eye are seen to originate at a common point, within the cross-hatched area, and the deviations of the experimental values from these lines do not (except for the value for Bramley's Seedlings in nitrogen, day 16, Fig. 3 *b*) exceed twice the standard deviation of an estimate, i.e. about 130 mg. carbon. Thus if the origin of the graphs is moved down about 100 units in Fig. 3 *a* and up about 50 units in Fig. 3 *b*, it is seen that the apples in these experiments lost carbohydrate steadily from the beginning of the periods of storage in air or nitrogen, and the rates of loss did not decline very much with time.

The loss of carbon is greater in nitrogen than in oxygen. This conserving effect of oxygen on respirable substrate, the 'Pasteur effect', has already been discussed fully for the present data (Fidler, 1948).

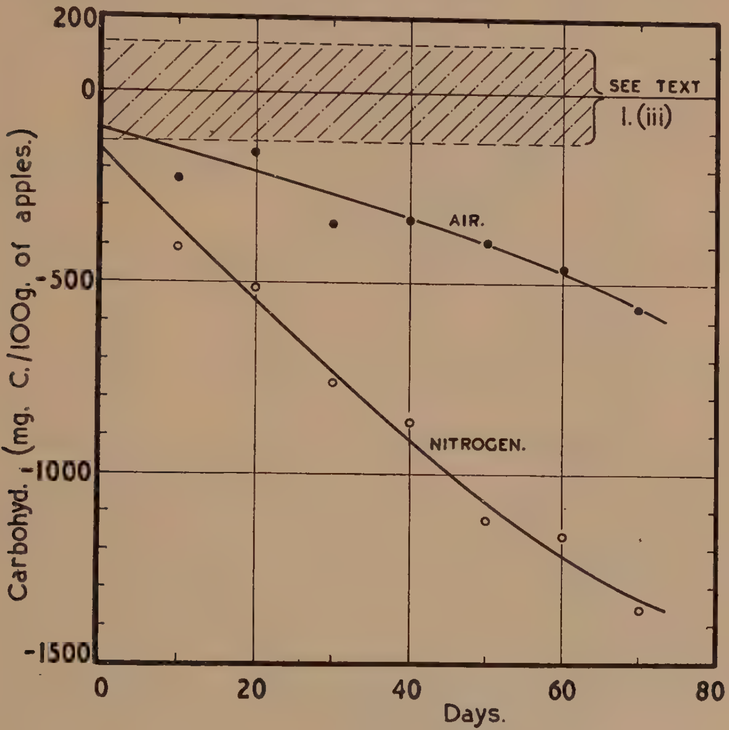

FIG. 3 *a*. Loss of carbohydrates from Sturmer Pippin apples in air and in nitrogen at 12° C., 1936–7.

iv. *Malic acid*. Simple plotting of the loss of acid against time gives an exponential curve, from which it is difficult to appreciate the factors governing the loss. Fig. 4 *a*, where the logarithm of the concentration of acid in the fruit is plotted against time, shows that in a typical experiment the rate of loss is proportional to the logarithm of concentration. This is not a new observation, but was first recorded by Haynes (1925). The facts that acid is lost in the absence of oxygen and that the rate of loss is the same in anerobiosis as in aerobic respiration are quite new and are surprising.[1]

[1] Unpublished data show that the rate of loss of acid from apples is the same in 5 per cent. oxygen+95 per cent. nitrogen, and in 65 per cent. oxygen+35 per cent. nitrogen, as in air. Further, the loss of acid from oranges is unaffected by the presence or absence of oxygen in the atmosphere (Fidler, 1937).

The analysis of the relationship between loss of acid and its concentration can be carried a stage farther. If the graphs for logarithm of concentration against time, for all experiments on a given variety at a given temperature, are plotted on the same scale, it is found that they all have the same slope. In Fig. 4 *b*, the points for three experiments on Sturmer Pippin apples at 12° C. are plotted together, using the same time units. Thus, at a constant temperature, the loss of acid from apples is related to the concentration of acid, and the same relationship holds over several seasons. Fig. 4 *b* also gives a graph for Bramley's Seedling apples at 12° C., the slope of which does not differ very markedly from that for Sturmer Pippins.

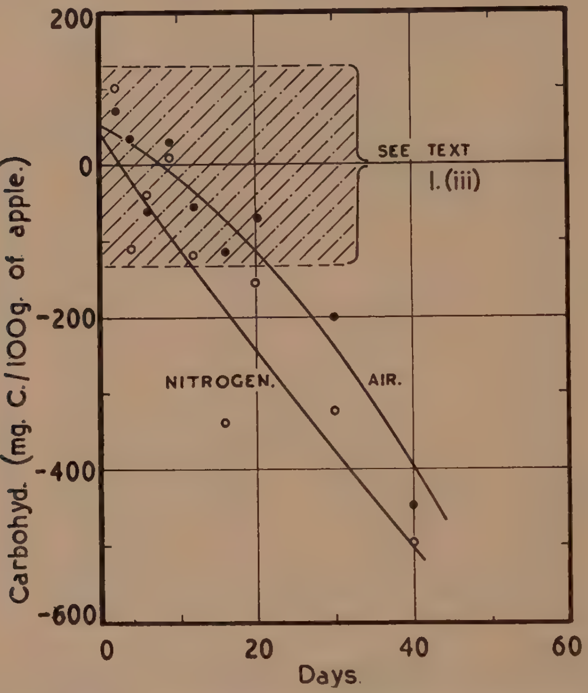

FIG. 3 *b*. Loss of carbohydrates from Bramley's Seedling apples in air and nitrogen at 12° C., 1935–6.

Bennet-Clark (1933), from evidence obtained from succulent plants and moulds, suggests that loss of acid takes place by reduction to an aldehyde, and Thomas (1949) considers that in succulent plants acid and carbohydrate are interconvertible, by transference of carbon dioxide, thus $C_6H_{12}O_6+2CO_2 \rightleftharpoons 2C_4H_6O_5$. This sort of reaction could explain why the presence or absence of oxygen does not affect the rate of loss of acid from apples. In this scheme decarboxylation of acid would liberate carbon dioxide and lead to production of carbohydrate, which would then suffer the same fate, in air or in nitrogen, as the rest of the carbohydrate. Certainly the facts mean that at least the initial stages of the reactions causing loss of titratable acidity are similar under aerobic and anaerobic conditions, but the reaction suggested by Thomas does not easily fit other facts, discussed in sub-section 2, where evidence is brought forward to show that malic acid is completely broken down to carbon dioxide both in air and in nitrogen.

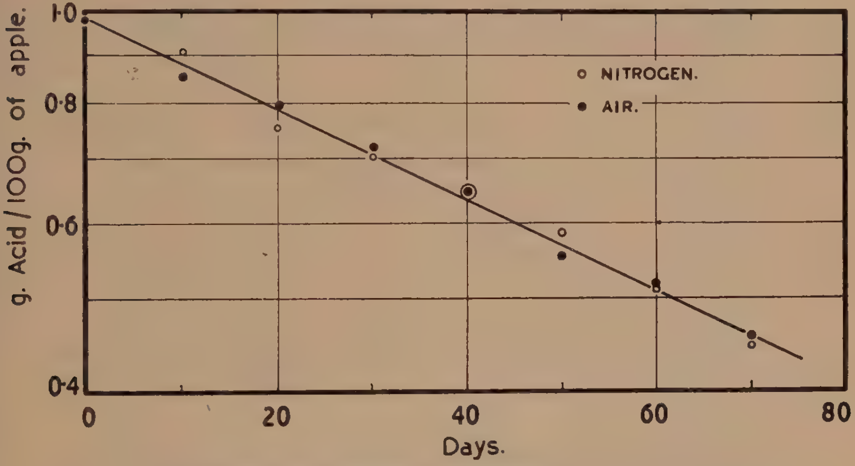

FIG. 4 *a*. Loss of acid from Sturmer Pippin apples in air and in nitrogen at 12° C., 1936–7.

2. *The correlation between the loss of substrate and the production of carbon dioxide and alcohol*

Before attempting to calculate figures for correlation it is necessary to consider what are the substrates of respiration. Carbohydrate and acid, which disappear regularly, must certainly be considered, and it has been suggested (e.g. Archbold and Barter, 1934) that the cell-wall material (represented in Table I by the alcohol-insoluble residue) may also contribute. Onslow, Kidd, and West (1931) have recorded that there are in the apple considerable quantities of unidentified alcohol-soluble substances; these may include respirable substrates, but in the absence of knowledge of their nature and amount an attempt must be made to see if the substances which have been estimated provide enough carbon to satisfy the requirements of the end products.

The next stage has been to plot the loss of carbon as carbohydrate, or carbohydrate plus acid, against the appearance of carbon as carbon dioxide plus alcohol, in order to determine whether the relationship between the quantities is linear. Typical graphs, for Expts. 144–5, are shown in Fig. 5. The straight lines through the points are drawn by eye, but it can be seen that the relationship between loss of substrate and accumulation of end products is linear. Similar graphs can be drawn for the other experiments, and it is only seldom that the points lie at a greater distance than twice the standard deviation from a straight line.

It has already been mentioned, for Fig. 3, that the line need not pass

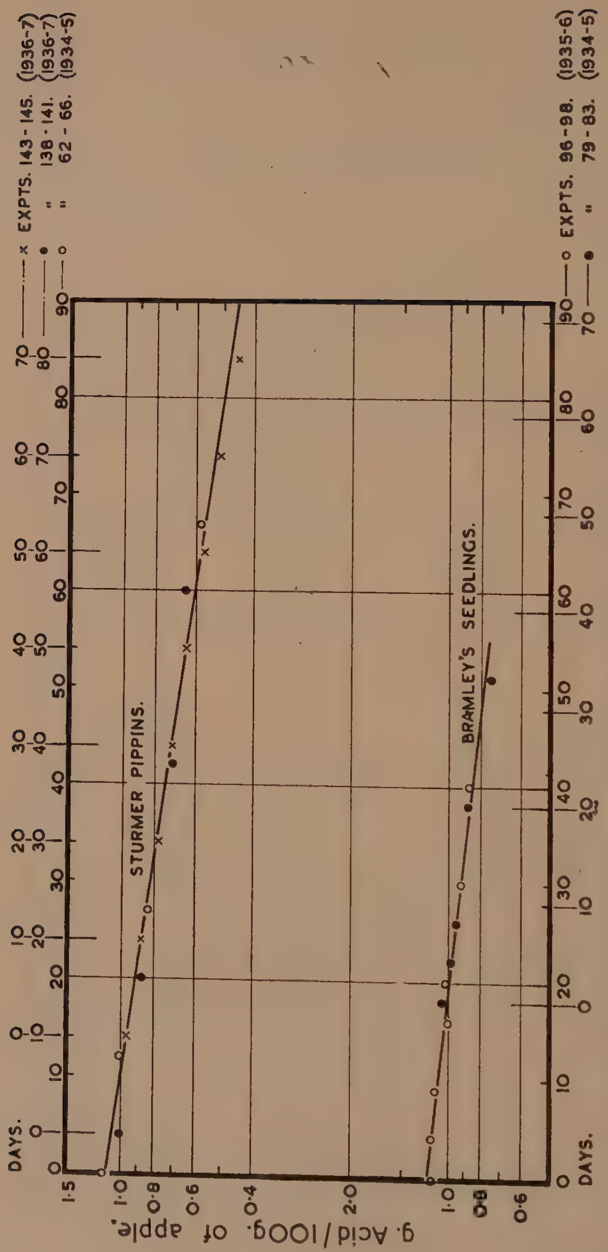

FIG. 4 *b*. Loss of acid from Sturmer Pippin and Bramley's Seedling apples at 12° C. in all seasons.

through the origin, because of errors in the initial estimates. These errors may be minimized, and all the data may be plotted on one graph, by recalculating the figures for each experiment, not from the initial value, but from the mean value.

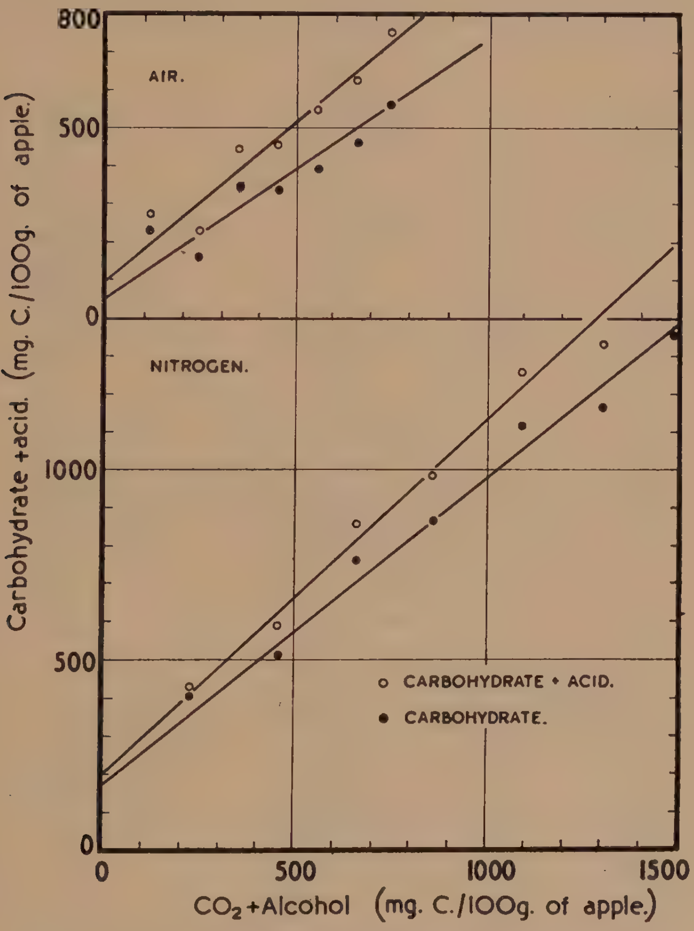

FIG. 5. The relationship between the loss of carbohydrate, or carbohydrate + acid, and CO_2 + alcohol, in apples stored in air or in nitrogen (Expts. 144–5. Sturmer Pippins 12° C.).

The notation used is as follows. In each experiment x_1, x_2,..., x_n are milligrams of carbon as carbon dioxide plus alcohol and $\bar{x}$ is the mean value (x_1 is the initial value, and is actually zero for carbon dioxide plus alcohol). Then $(x_1-\bar{x})$, $(x_2-\bar{x})$,..., $(x_n-\bar{x})$ are the deviations from the mean value. Similarly $(s-\bar{s})$ and $(u-\bar{u})$ are the deviations from the mean values for loss of carbon as carbohydrate, and as carbohydrate plus acid, respectively.

In Figs. 6 *a* and *b* all the values for all the samples have been plotted together; in Fig. 6 *a* the end products (x) are plotted against carbohydrate (s), and in Fig. 6 *b* they are plotted against carbohydrate plus acid (u). It is clear that the best line through the point, on either graph, is a straight line through the origin. In other words, the relationship is a simple linear function. This means that straightforward methods may be used to calculate regression and correlation coefficients. Before doing so, however, the qualitative observation may be made that in Fig. 6 *a* the loss of substrate is inadequate to account for the end products, while in Fig. 6 *b* the equivalence is very good. (The straight lines drawn through the points are the regression lines, calculated as below.)

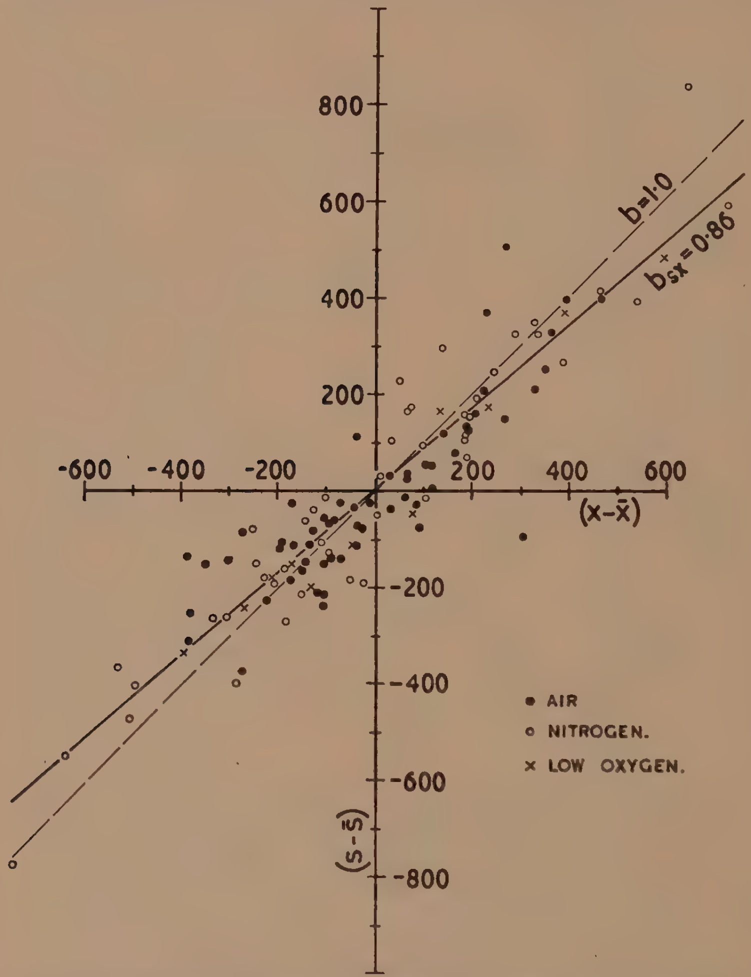

Fig. 6 *a*. The relationship between the loss of substrate (carbohydrate, $s-\bar{s}$) and the appearance of end products (CO_2 + alcohol, $x-\bar{x}$) in apples stored in air or in nitrogen.

The possibility that the material of the cell-wall may have contributed to the substrate has been mentioned. The loss of this material (the alcohol insoluble residue) was irregular and small in amount, in most experiments, but in Expts. 1 and 53 it appeared to be regular and greater in magnitude than the sampling error. Additional statistical coefficients have been calculated for these two experiments, taking the residue into account. The notation used

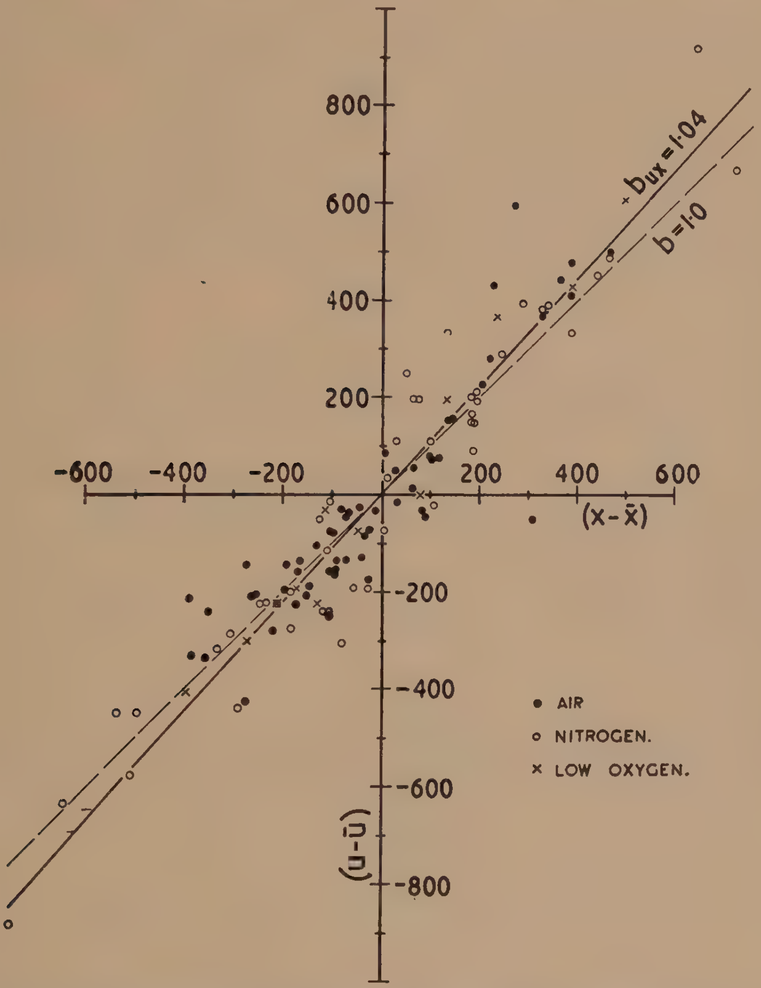

Fig. 6 *b*. The relationship between the loss of substrate (carbohydrate + acid, $u-\bar{u}$) and the appearance of end products (CO_2 + alcohol, $x-\bar{x}$) in apples stored in air or in nitrogen.

is y for carbohydrate plus residue and z for carbohydrate plus acid plus residue.

The formulae for the statistical coefficients are:

(*a*) the correlation coefficient, r, i.e. the correlation between x and s:

$$r_{xs} = \frac{\sum (x-\bar{x})(s-\bar{s})}{\sqrt{\{\sum (x-\bar{x})^2 . \sum (s-\bar{s})^2\}}};$$

(*b*) the regression coefficient, *b*, e.g. the increment in *s* for unit increment in *x*:

$$b_{sx} = \frac{\sum(x-\bar{x})(s-\bar{s})}{\sum(x-\bar{x})^2};$$

(*c*) the standard deviation of the regression coefficient:

$$\sigma_b = \sqrt{\left\{\frac{(1-r_{xs}^2)\sum(x-\bar{x})(s-\bar{s})}{\sum(n-1)-1\,.\,\sum(x-\bar{x})^2}\right\}},$$

where r_{xs} is the correlation coefficient, and $n =$ the number of observations, including the initial, in any experiment. The limits of, for example, b_{sx} may be found from σ_b, using the *t* test, and σ_b may also be used to compare two regression coefficients, by the *Z* test. The results of the statistical examination of the data are given in Tables II and III.

TABLE II

The relationship between carbon dioxide plus alcohol (*x*), *and carbohydrate* (*s*) *or carbohydrate plus acid* (*u*), *in the respiration of apples*

	$x:s$			$x:u$			
Group	r_{xs}	b_{sx}	σ_b	r_{xn}	b_{ux}	σ_b	Degrees of freedom
All experiments in air .	0·821	0·724	0·09	0·879	0·975	0·087	47
Experiments in low concentrations of oxygen .	0·963	0·898	0·087	0·974	1·15	0·077	9
Experiments in nitrogen .	0·963	0·923	0·064	0·962	1·06	0·069	36
All experiments . .	0·920	0·858	0·063	0·937	1·04	0·062	94

TABLE III

The relationship between carbon dioxide plus alcohol (*x*), *and carbohydrate* (*s*), *carbohydrate plus acid* (*u*), *carbohydrate plus residue* (*y*), *or carbohydrate plus residue plus acid* (*z*), *in the respiration of Sturmer Pippin apples* (*Expts.* 1 *and* 53 *only*)

Degrees of freedom = 11.

	$x:s$	$x:u$	$x:y$	$x:z$
r . .	0·921	0·935	0·922	0·940
b . .	0·864	1·11	1·08	1·34
σ_b . .	0·102	0·108	0·115	0·114

The first point to note, from Tables II and III, is the high value of the correlation coefficient; $P < 0{\cdot}01$. It is noteworthy, too, that in Table II the values of *r* are somewhat higher for substrate *u* than for substrate *s*. Further, the regression of carbohydrate (*s*) on carbon dioxide plus alcohol (*x*) is always less than unity (it is significantly so when all the experiments are grouped together), whereas the regression of carbohydrate plus acid (*u*) on *x* is very nearly unity. Thus in all the groups of experiments in Table II, for each unit of carbon appearing as carbon dioxide plus alcohol, one unit is lost as carbohydrate plus acid, but only 0·8 units as carbohydrate alone; the loss of carbohydrate plus acid supplies the total carbon needs of the end products.

Considering now the data of Table III, where values are presented for substrates u and s derived from the same samples used to calculate y and x, it is seen that b_{ux} is not significantly different from unity. (The actual calculated value, 1·11, has a greater error than the value 1·04 shown in Table II, because of the much smaller number of samples.) The value of b_{yx} is also approximately unity, but it has a somewhat smaller correlation coefficient, and a larger error, than b_{ux}. When acid is included as well as the residue (b_{zx}), the regression coefficient significantly exceeds unity. It is thus probable that in these two experiments, where there was a measured loss of alcohol insoluble residue greater than the sampling error, the loss was not directly contributing to respirable substrate. There were no estimates of those alcohol-soluble fractions which escape analysis, but it is possible that the cell-wall material passed into these fractions.

In work of this nature on biological material, in which one of the possible substrates, namely, carbohydrate, is subject to large combined errors of sampling and estimation, and in which all the trends of the metabolites are regular in form, high values of correlation coefficients and unity regression lines do not necessarily imply strict connexion between substrate and end product. It is not unreasonable, however, to conclude from the above data that apples respire carbohydrate and acid, in air or in nitrogen, and that the total loss of carbon from the substrates appears in the end products.

This conclusion does not agree with that of Archbold and Barter (1934), who, using the half-apple method of Lall (1934), concluded that the loss of carbon dioxide from apples in air was about 17 to 30 per cent. less than the loss of acid plus carbohydrate. There are good grounds for thinking that their conclusion is not correct. One of their experiments began on 20 November and lasted for 7 weeks in air at 12° C. Another experiment which lasted for 4½ weeks began on 20 February; these Bramley's Seedling apples were said to be 'nearing the end of their storage life'. No estimates of the production of ethyl alcohol were made. If it is assumed that these senescent apples were producing alcohol in air, about 0·1 per cent. would be needed to balance the excess loss of substrate over production of carbon dioxide. This is not an improbable amount for senescent Bramley's Seedlings. Thus, Fidler (1933) found that Bramley's Seedlings removed from 3° C. in March with an alcohol content of 0·04 per cent., produced a further 0·18 per cent. during 100 hours in air at 23° C. In other words, it must be remembered that, even in air, carbon dioxide is not necessarily the only end product. The necessity of estimating ethyl alcohol as well as carbon dioxide in order to obtain a true picture of respiratory processes has already been pointed out (Thomas and Fidler, 1933).

The conclusion drawn in the present paper receives support from measurements of the respiratory quotient. In work on the metabolism of acetaldehyde (to be described in a subsequent paper) the respiratory quotient of large samples of apples in air was determined. In a typical experiment the measured value was 1·16. When corrections were made for oxidation of the acid which had disappeared, and for the alcohol which was produced, the residual respiratory quotient for oxidation of sugar was 0·98. The same figure, which is very near to the theoretical value of 1·0, was obtained as the mean value for six experiments.

3. *The anaerobic respiration of carbohydrate and acid by the apple*

Having established that the carbon dioxide and ethyl alcohol produced by apples under anaerobic conditions is equivalent to the carbohydrate and acid lost, it remains to be seen whether the observed relations between the amounts of carbon dioxide and alcohol can be accounted for.

It was noticed that when the quotient CO_2/C_2H_5OH was found to be high, the subsequent analyses showed that there was a high rate of loss of acid relative to carbohydrate. Considering two examples from Table I, in which the loss of acid was of the same order, namely, about 190 mg. of carbon, it is seen that in sample 1 J, where the loss of sugar was 720 mg. carbon, the quotient has a value of 100/43, whereas in sample 145 G the loss of sugar carbon was 1,356 mg., and the quotient was 100/71. Such a change would be expected whether malic acid broke down to give carbon dioxide plus alcohol, and even more if it broke down to give carbon dioxide only.

The loss of carbon as carbon dioxide arising from the normal reaction for alcoholic fermentation of carbohydrate may be calculated from the alcohol number. Subtracting these values from the total carbon dioxide figure, values are obtained for 'extra' carbon dioxide, produced by some process other than fermentation. The resultant 'extra' carbon dioxide figures (v) and the loss of acid (Ac) can be brought together on one graph (Fig. 7 *a*) by adopting the device used above for substrate and end product, i.e. by calculating ($v-\bar{v}$) and ($Ac-\bar{Ac}$). The dotted line on Fig. 7 *a* is the line for equivalence of Ac and v; the solid line is the calculated value of the regression coefficient, b_{Acv}. Fig. 7 *b* gives the results for the two experiments (1 and 141) in which nitrogen was used straight from the cylinder, without having the small amount of oxygen (0·2 to 0·6 per cent.) removed. It is clear that in both graphs the relationships are linear.

A similar analysis may be done of the values for carbohydrate (s) and the carbon dioxide plus alcohol derived from fermentation (f). This is not given graphically, for since $Ac:v$ is linear (Fig. 7 *a* and *b*) and $x:u$ is linear (Fig. 6 *a* and *b*), it follows that $s:f$ must also be linear.[1] Results are given in Table IV.

The correlation (r) between fermentation and loss of carbohydrate and between the production of 'extra' carbon dioxide and the loss of acid is very high. The probability that the correlation is a matter of chance is negligible. When the regression coefficients are considered together with the values of σ_b, it is seen that, except in Expts. 1 and 141, all the carbohydrate is fermented according to the usual equation, and each unit of carbon as 'extra' CO_2 is accompanied by one unit of carbon lost as acid.[2]

[1] $f+v = x$ and $s+Ac = u$.

[2] In Expts. 1 and 141, presumably some of the 'extra' CO_2 is derived from oxidation of sugar, by the trace of oxygen present in the gas stream.

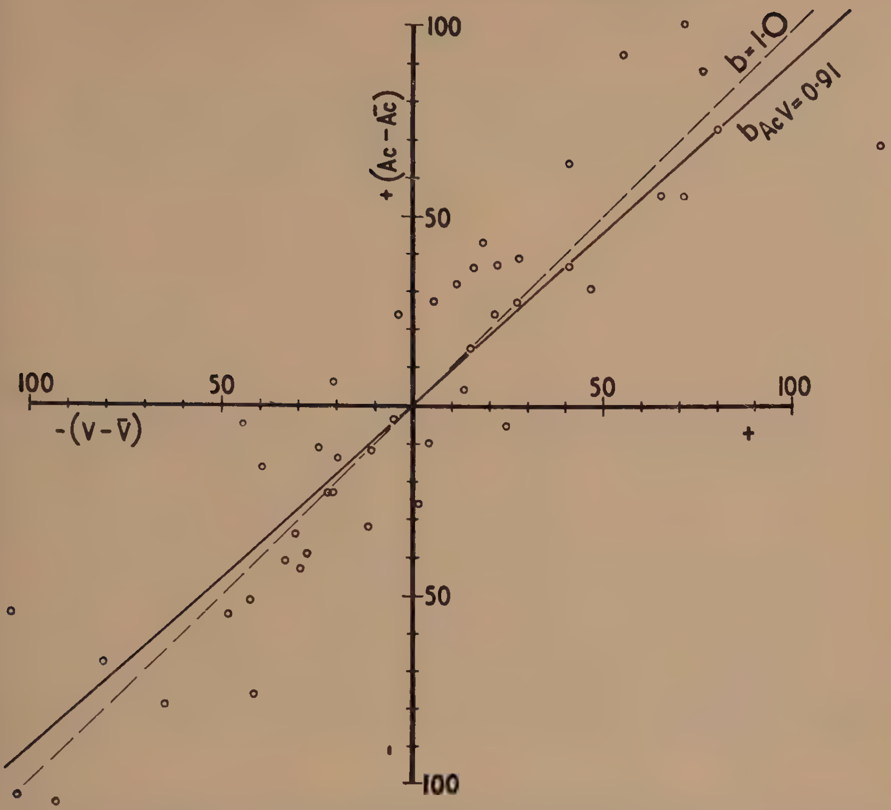

FIG. 7 *a*. The connexion between the loss of acid (*Ac*) and the production of 'extra' CO_2 (*V*) in the anaerobic respiration of apples.

TABLE IV

The correlation between (1) *fermentation* (*f*) *and the loss of carbohydrate* (*s*), *and* (2) *the production of 'extra' carbon dioxide* (*v*) *and the loss of acid* (*Ac*). *Apples under anaerobic conditions*

	f:*s*			*v*:*Ac*			
Group	r_{fs}	b_{sf}	σ_b	r_{vAc}	b_{Acv}	σ_b	Degrees of freedom
Sturmer Pippin . .	0·975	1·02	0·066	0·900	0·921	0·051	21
Bramley's Seedling . .	0·904	1·25	0·091	0·869	0·874	0·047	14
All experiments in pure nitrogen . . .	0·965	1·04	0·061	0·923	0·913	0·035	36
Experiments 1 and 141 .	—	—	—	0·988	0·835	0·024	9

This result is an important one; it is strong evidence that the anaerobic respiration of carbohydrate by a higher plant has the same end products as the fermentation of sugar by yeast, and that the summary equation

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

holds good for carbohydrate.

The summary equation for all respiratory reactions in nitrogen may be written:

$$nC_6H_{12}O_6 + mC_4H_6O_5 \rightarrow xCO_2 + yC_2H_5OH.$$

The fact that $x > y$ is because carbon dioxide is being produced from acid without any accompanying production of alcohol; only when m is zero does $x = y$.

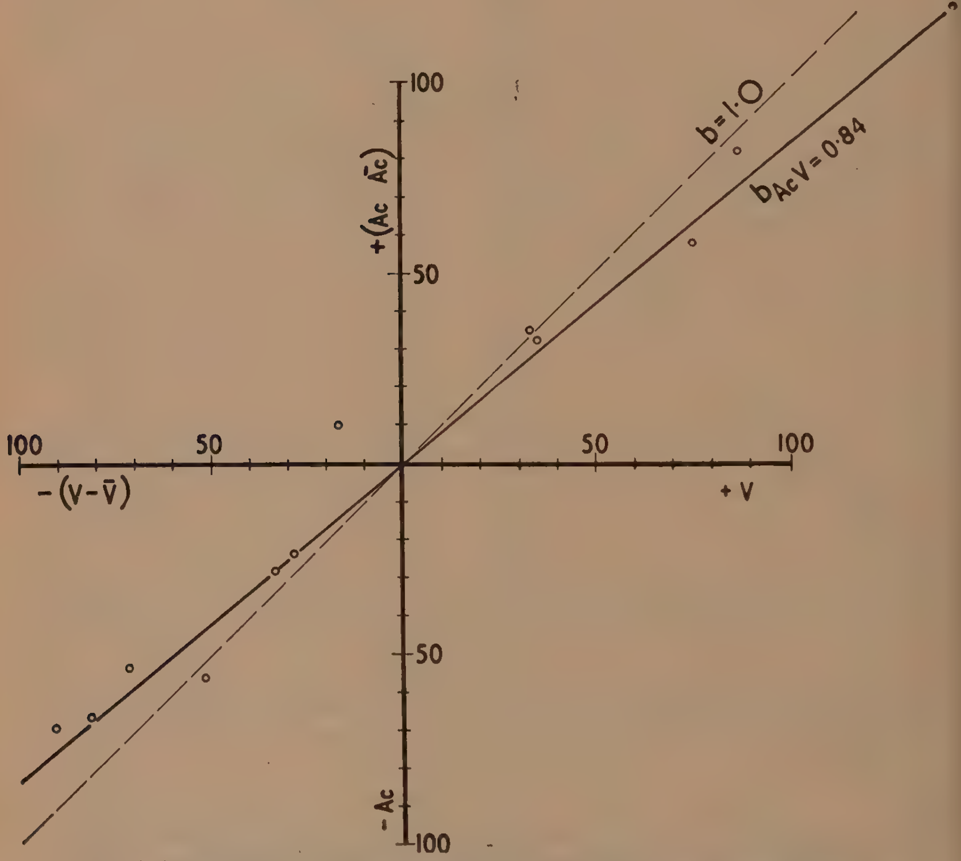

FIG. 7 *b*. The connexion between loss of acid (*Ac*) and the production of 'extra' CO_2 (*V*) in apples stored in low concentrations of O_2 (0·2–0·6%) (Expts. 1 and 141).

The production of the 'extra' carbon dioxide under anaerobic conditions cannot be a chance effect, due to traces of oxygen in the nitrogen used. If this were so, it would mean not only that the utilization of oxygen from gas mixtures containing very low concentrations was governed by the rate of loss of acid, but would require more oxygen than the gas streams could have supplied (except in Expts. 1 and 141). For example, it has been calculated that the nitrogen used in Expts. 53, 59, 74, and 145 would have had to contain at least 0·1 to 0·25 per cent. of oxygen to supply an oxidation system producing the 'extra' carbon dioxide. This is impossible.

How, then, is the production of the 'extra' carbon dioxide bound up with the loss of acid?

Reverting to Thomas's suggested scheme (loc. cit.) for interrelation of acid and carbohydrate, it may be expected that, in air, the first stages in the catabolism of malic acid would be:

$$\text{Malic acid} \rightarrow \text{Oxalacetic acid} + 2\text{H} \qquad (1)$$

$$\text{Oxalacetic acid} \rightarrow \text{Pyruvic acid} + \text{CO}_2 \qquad (2)$$

Subsequent stages may be either:

$$\text{Pyruvic acid} + 2\text{H} \rightarrow \text{Triose} \qquad (3)$$

or, $$\text{Pyruvic acid} + 2\text{H} \rightarrow \text{CO}_2 + \text{H}_2\text{O}. \qquad (4)$$

In this scheme the triose would be built back to form a C_6 respirable sugar. While this whole sequence of reactions may occur in aerobic conditions it is improbable for apples in nitrogen, for it is difficult to see why the C_3 or C_6 sugar should not be fermented.

The simplest and most direct explanation of the present results, where all the carbon of the malic acid appears as carbon dioxide, is oxidation. If the carbon atoms of the 'extra' carbon dioxide are derived from malic acid, this is the only explanation. There is, however, one other possibility, that is, that the malic acid is in equilibrium with some unestimated substance, X, and that both malic acid and X are decarboxylated, with attendant production of unidentified substances. This second hypothesis is very unsatisfactory; it has too many unknown factors.

Neither is the oxidation hypothesis entirely satisfactory in view of the high demand for oxygen. For example, oxidation of the acid in Expt. 145 would require 384 mg. of oxygen, or about 0·16 ml./100 g./hour. Hence the oxidation of malic acid in nitrogen would involve the formation of large quantities of highly reduced compounds; so far no evidence of the production of such substances has been obtained. Apples produce long-chain paraffins in the waxes which develop late in storage, and the production of the precursors of these substances may involve reactions which free oxygen. Beyond this it is impossible to go, with present knowledge, but researches in progress on the nature of such waxy materials in apples may yield further information.

My thanks are due to Dr. J. Wishart, for advice on the statistical treatment, to Miss D. G. Griffiths and Miss N. A. Potter, who did the carbohydrate analyses in Expt. 1, and to Mr. C. J. North, who did all the other carbohydrate and acid estimations.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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Apparatus for the Measurement of Gaseous Conditions inside an Apple Fruit

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It was noticed in the course of studies of the behaviour of apples which had been coated with various oil-water emulsions (see e.g. Hulme, 1949) that some of the fruits exhibited symptoms of physiological diseases typical of fruit stored in low concentrations of O_2 and high concentrations of CO_2 (see Kidd and West, 1927). This suggested that the effect of skin-coatings on the storage behaviour of the fruit was related to their effect on the composition of the atmosphere within the fruit and that to study these effects it was necessary to measure the content of CO_2 and O_2 within the fruit.

Considerable variation in behaviour was observed in fruit treated with the same coating. Hence it was only by examining several hundred apples in a relatively short time that it was possible (*a*) to obtain some indication of the effect of any given treatment on the extent of the variation in the composition of the internal atmosphere (I.A.) from fruit to fruit, and (*b*) to obtain a 'statistical' average for the composition of the I.A. for any one treatment.

An essential criterion, therefore, of any method for the determination of the I.A. of coated fruits was that it should be rapid and capable of dealing with several apples a day. A high degree of accuracy was not essential since variations of the order of 0·2 per cent. of O_2 and CO_2 would not be likely to influence the storage behaviour of the apples to any marked extent. In addition, the method must not destroy the fruit or bring about any serious interference with its metabolism. This made inadvisable the use of such methods as those of Kidd (1937) and of Magness (1920) since such methods employ cylindrical plugs of tissue removed from the fruit. These and other methods—notably that of Wardlaw and Leonard (1939)—have been discussed recently by W. H. Smith (1947).

As a starting-point in the present work the apparatus of W. H. Smith (1947) appeared to offer the best possibilities, and the methods to be described are, essentially, modifications of Smith's technique.

Early experiments showed that in skin-coated fruits the combined concentration of CO_2 and O_2 often fell far short of the 20–21 per cent. found in non-senescent, uncoated fruits. In these cases it would appear that the pressure within the fruit might fall below atmospheric pressure.

It was therefore decided to examine, in addition to the composition of the internal atmosphere (I.A.), the internal pressure (I.P.) developed in coated apples. Apart from the direct interest afforded by the measurement of I.P., it was thought that the change in pressure observed following the application

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of a skin-coating might provide a rapid indirect method for measuring the extent of the change produced by the coating in the gaseous conditions inside the fruit.

Two types of apparatus were therefore developed: one for the rapid routine analysis of the I.A. of fruit taken from storage experiments, and one for the simultaneous measurement of the I.A. and I.P. of fruit sprayed with various skin-coating liquids *in situ* on the apparatus. It is the purpose of the present paper to describe these two forms of apparatus and to give examples of the results obtained by their use.

DESCRIPTION OF THE APPARATUS

I. *The apparatus for the routine analysis of the internal atmosphere of an apple.* (Apparatus I)

The construction of this apparatus is shown in diagrammatic form in Fig. 1. The apple is placed on the cast aluminium platform, P, after a little warm vaseline had been run on to the platform round the hypodermic tubing (0·8 mm. diameter) B through which passes a close-fitting stainless wire. This wire projected a fraction of a millimetre above the upper tip of the tubing, which is cut at an angle of 45° to give a sharp point for piercing the fruit, and it prevented tissue or juice entering the hypodermic tubing during the lowering of the fruit. The vaseline makes a seal between the apple and the platform, the apple being held in place by the three movable arms (e.g. H) which are rubber-covered. The hypodermic tubing is held rigid in a stainless steel tube, F, by means of the rubber bung, D, and, at the bottom end, by means of the 'cup' of a normal hypodermic needle screwed into tube F and to which the hypodermic tubing is sweated. The hypodermic tubing projects about 6 mm. beneath this cup, the projecting tubing piercing the centre of a hemispherical piece of rubber (made from a small rubber bung with the lower surface ground to a hemisphere) so that it projects about 1 mm. below the bottom of the hemisphere. When the sampling tube, S, is in position for the withdrawal of a sample of gas, the rubber hemisphere, E, makes a gas-tight seal with the cup of the sampling tube.

When the apple is being placed on the platform, tube F is prevented from moving by means of the clip C which may be tightened on the tube by means of a knurled-headed screw. The height of B above the platform is adjusted to the approximate height of the centre of the apple before the latter is placed in position.

Smith (1947) has demonstrated the existence of a gradient in the composition of the internal atmosphere from the centre to the periphery of an apple. For a comparison of the I.A. of a series of apples it is necessary, therefore, to withdraw a sample of gas for analysis from approximately the same point in each fruit. The core-cavity is a convenient region for this purpose, since from this position there is little risk of juice entering, and possibly blocking, the hypodermic tubing; this position is, of course, inadmissible for 'open eye'

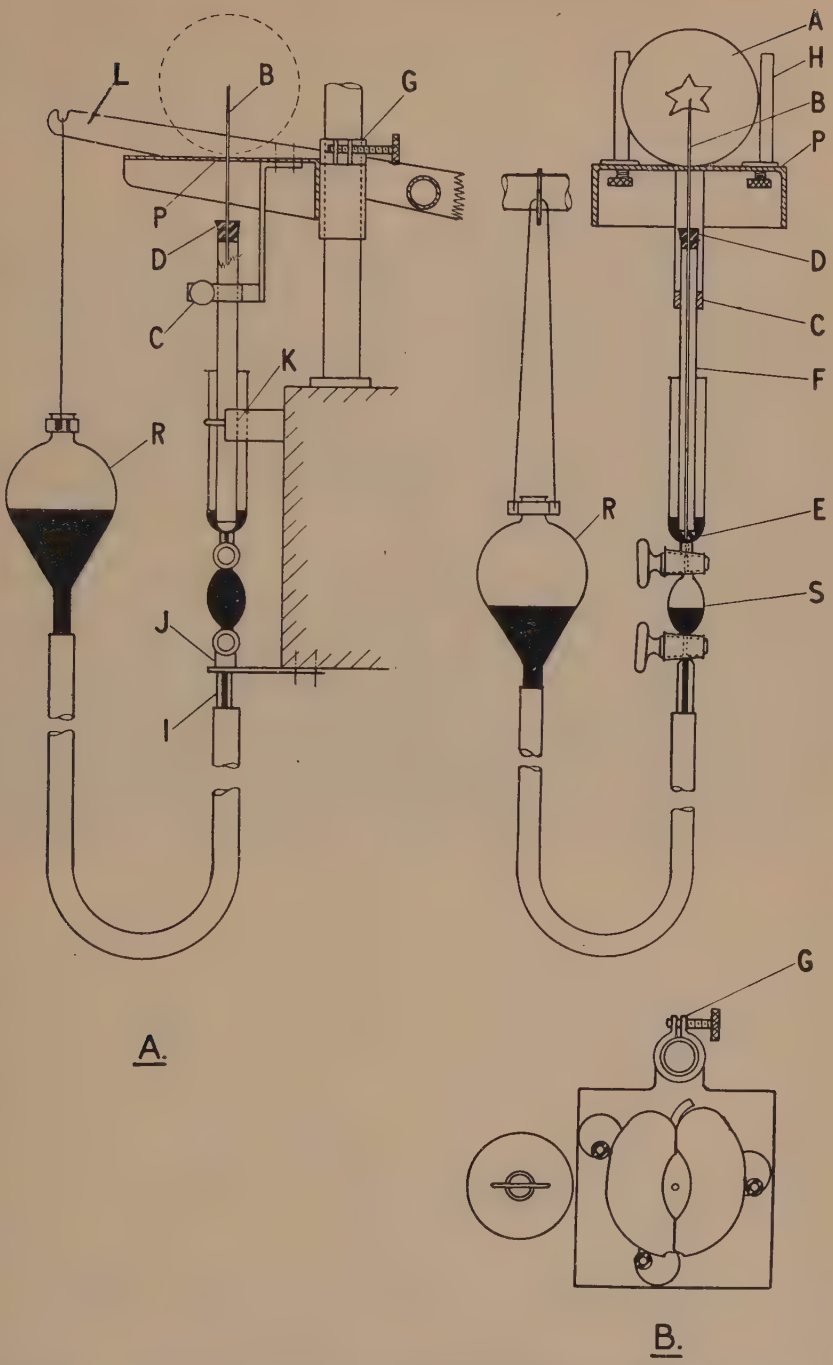


FIG. 1. Diagrams of the apparatus (apparatus I) for the routine determination of the I.A. of apple fruits. Side and front elevations of apparatus and plan of apple platform.

varieties of apples. The point of B should therefore be in the core-cavity of the apple. To test this when the apple is in position the wire passing through B is moved gently up and down. After a little experience has been gained the operator can tell from the 'feel' of the wire if the tip of B is in the correct position. If this is not so, clamp C is loosened and tube F moved up or down slightly until B projects into the core cavity.

The sampling tube is held in position on a wooden stand as shown in Fig. 1 *a*. The weight of the tube is taken by a metal bracket through which the capillary tube I of the sampling tube passes. The small rubber bung J through which the capillary tube passes acts as a flexible buffer. The sampling tube is prevented from falling forward before the apple-platform is lowered by a spring-loaded clip which holds it in the U-shaped bracket K.

When commencing the withdrawal of a sample of gas from the apple the mercury reservoir is raised until, with both taps of the sampling tube open, the mercury level in the cup of the sampling tube is about 5 mm. above the top tap. The quantity of mercury in the reservoir should be such that at this position the wire sling holding R just hooks into the slot shown at the end of a movable arm.

The apple chamber is then lowered on its pillar until the rubber tip E rests firmly in the bottom of the cup of the sampling tube. The mercury in the cup will then have risen to cover E completely, as shown in Fig. 1 *a*. The upper platform is held down firmly by means of the clip G which is clamped to the pillar by tightening the knurled screw. The pressure exerted by the compressed rubber of E and J maintains an adequate seal between E and the bottom of the cup of the sampling tube. The air-tightness of this seal may be tested by opening the two taps of the sampling tube and gently lowering the reservoir about 1 cm. If the seal is not satisfactory mercury will flow from the cup of the sampling tube down through the top tap of the sampling tube.

The reservoir holder is next replaced in the slot as shown, the electric motor driving the gear train which lowers the arm is switched on, and, as the reservoir R falls, gas is withdrawn from the core-cavity of the apple into the sampling tube which has a capacity of about 1 ml. Fig. 1 *b* illustrates a sample of gas in process of collection. Only very occasionally has the vaseline seal between the apple and the platform broken down, and it has not been found necessary to use the rubber gasket sealed to the surface of the apple as advocated by Smith (1947). The speed with which the sample of gas is collected can be varied by means of a variable resistance in circuit with the electric motor. Periods of 1–24 hours have been used; the effect of the rate of removal of the sample will be discussed later. A trip-switch is incorporated in the circuit of the motor so that the latter is switched off when the mercury in the sampling tube is just about to enter the bottom tap of the sampling tube. It is important that the bore of the tap and the capillary tube below it remain full of mercury, otherwise a leakage of air into the sampling tube through the tap will occur when the gas in the sampling tube is sucked into the Van Slyke manometric apparatus (see later) for analysis.

For the routine analysis of the I.A. of a population of apples, six of the gas-collecting units are fixed to one wooden framework, all the mercury reservoirs being lowered simultaneously by arms mounted on a common bar. The arrangement of this multiple apparatus will be seen from the photograph in Fig. 2.

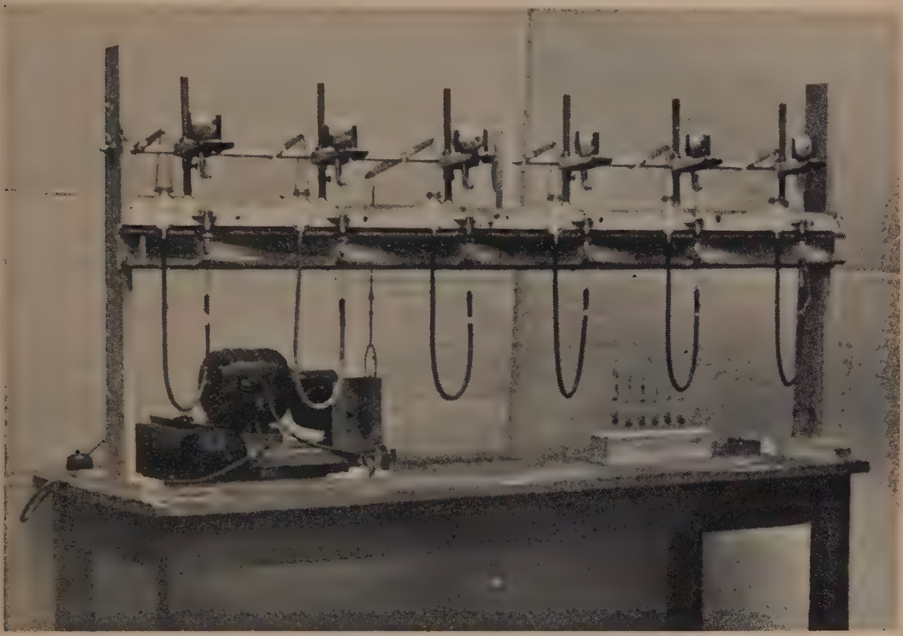


FIG. 2. Photograph of assembly of six units in operation.

II. *The apparatus for the measurement of both the I.A. and the I.P. of an apple under various external conditions.* (Apparatus II)

The apparatus is shown diagrammatically in Fig. 3 and a photograph of the actual apparatus in use is shown in Fig. 4. For special purposes a more elaborate form of the chamber surrounding the apple was constructed in Perspex; this is illustrated in Fig. 5.

From Fig. 3 it will be seen that the method of withdrawing a sample of gas from the core-cavity of the fruit for analysis is the same as that used in the apparatus described in the previous section. To enable the internal pressure of the fruit to be measured, the hypodermic tubing with its outer supporting tube bifurcates below the apple-platform. The right-hand bifurcation, G, may be sealed into the manometer, M 1, by lowering the chamber until the rubber hemisphere on the end of G fits closely into the cup of the left-hand limb of the manometer. Since it is impossible to arrange, by simply lowering the platform, that both rubber hemispheres make perfect seals in their respective cups, a screw device (having the action of a rack and pinion) is provided

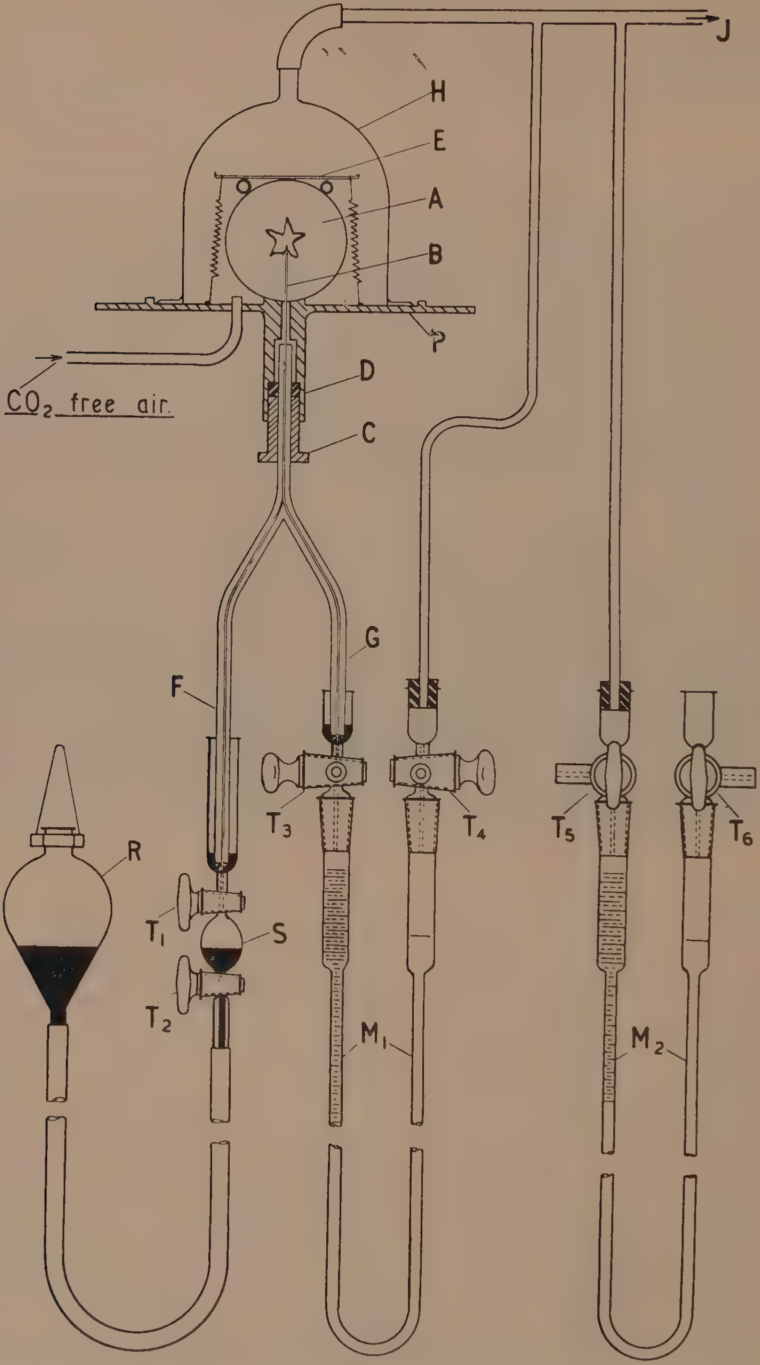


FIG. 3. Diagram of the apparatus (apparatus II) for the simultaneous measurement of the I.A. and the I.P. of an apple.

whereby the sampling tube can be raised slightly so that the cup presses against the rubber hemisphere on the left-hand bifurcation of the hypodermic tubing. This device is omitted from Fig. 3 to simplify the presentation. Mercury is placed in both cups when the hemispheres are in position to act as an additional seal.

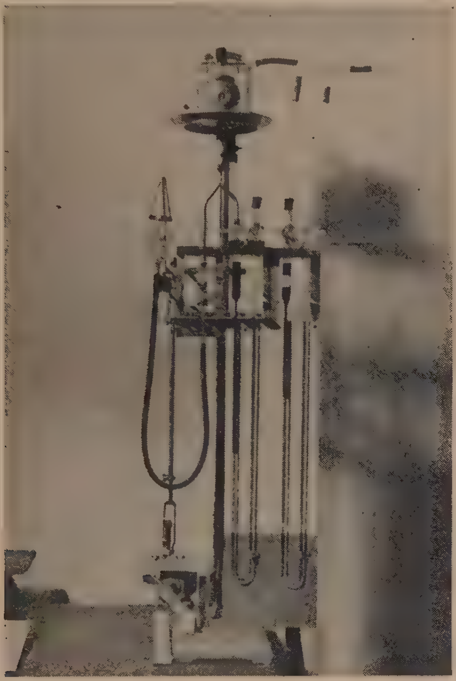


FIG. 4. Photograph of apparatus II in operation.

The two manometers M 1 and M 2 are of the differential type (Ower, 1927), being filled with two immiscible liquids of about the same density. The shaded portion in the left-hand limbs of the two manometers consists of benzyl alcohol containing sufficient aniline black to make the meniscus between the two liquids easily visible. The liquid filling the remainder of this limb and most of the right-hand limb consists of aqueous calcium chloride (sp.gr. 1·20).

The amount of the two liquids must be carefully adjusted so that there is a minimum of dead-space between the benzyl alcohol and the bottom of the glass cone below tap T 3, and also so that at the extreme travel upwards of the benzyl alcohol (which should be just to the bottom of the glass plug below T 3) the level of the calcium chloride in the right-hand limb does not enter the curvature where the wide section of the limb is joined to the lower narrower section. If this latter does occur, of course, the magnification factor of the manometer (which is a function of the relative diameters of the wide and narrow sections) will not remain constant. The magnification factor of the manometers used was 3·4.

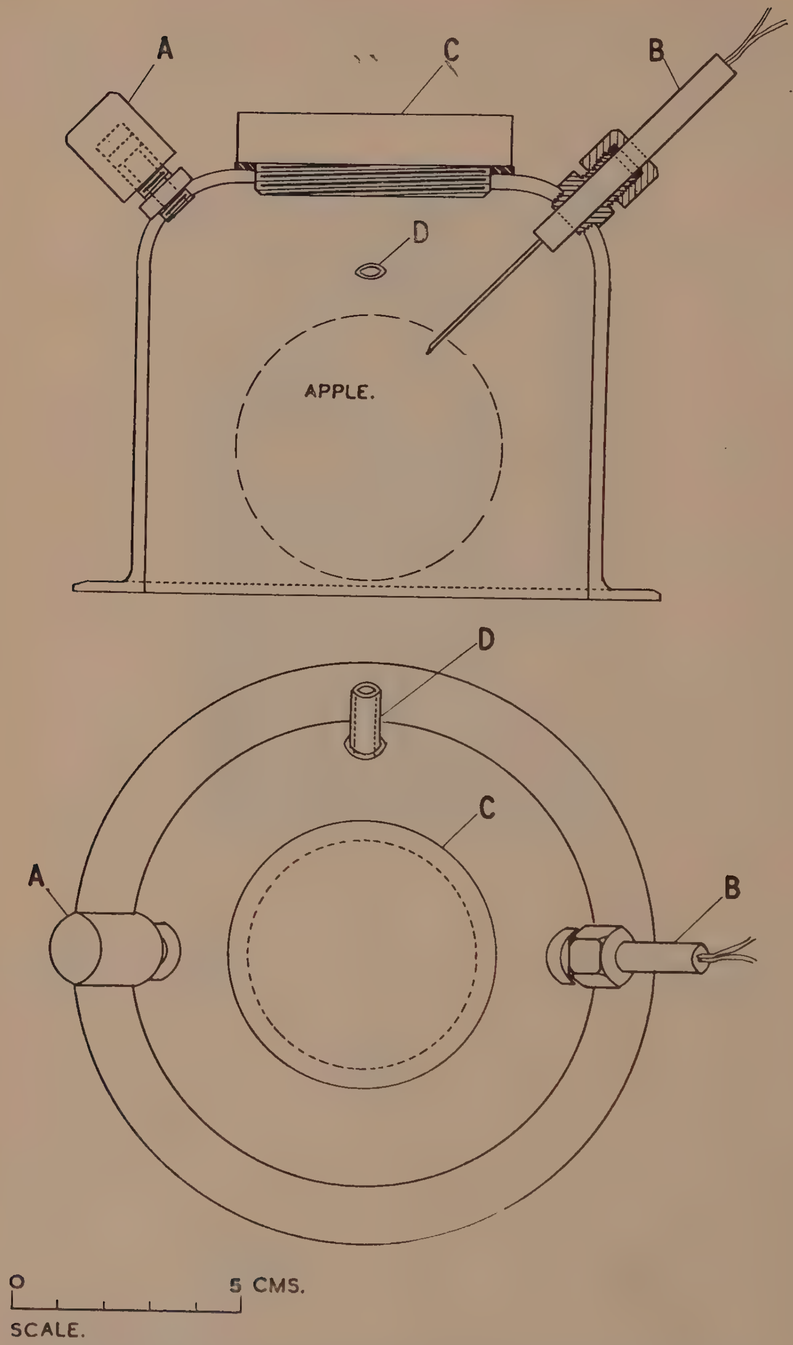


FIG. 5. Diagram of special Perspex apple chamber for use with apparatus II.

The glass (or Perspex) chamber H, which is sealed to the platform by means of a mixture of vaseline and medicinal paraffin between the two faces, has a hreefold purpose: (i) it enables the CO_2 output of the fruit to be measured; ii) it enables the effect on the I.A. of the apple of subjecting it to external gases to be measured; and (iii) it makes possible, by subjecting the apple to a small negative pressure (the extent of which pressure may be measured by neans of manometer M 2), the measurement of the permeability to gases of he tissue between the outside of the apple and the core-cavity. By a comparison of this permeability before and after the application of a coating to the surface of the apple a measure is obtained of the change in permeability to gases (strictly, air) which results from the coating.

By means of the Perspex chamber illustrated in Fig. 5 it is possible to neasure the temperature of the fruit at any depth below its surface by the nsertion of airtight sleeves (as at B, in the figure) carrying thermocouples ealed into the tip of a hypodermic needle. The airtight cap C may be removed o spray portions of the surface of the apple with a skin-coating. To obtain a atisfactory coating over the whole apple the complete chamber must be emoved.

Analysis of the gas collected in the sampling tubes

The method used to determine the CO_2 and O_2 content of the gas collected rom the core-cavity of the apple with both forms of apparatus was the Van Slyke manometric apparatus. The method was used exactly as described by Van Slyke and Neill (1924) with the exception that 1:2:4-triacetoxybenzene 10 g. dissolved by warming in a closed bottle with 100 ml. of water containing 3·5 g. KOH) was used instead of sodium hydrosulphite for the absorption of oxygen. Absorption of oxygen was complete in 4 minutes' shaking of the eaction chamber.

The gas was transferred from the sampling tubes to the Van Slyke apparatus y placing a rubber hemisphere, similar to the one used on the end of the ypodermic tubing, on the drawn-out tip of the capillary of the sampling tube the capillary remains full of mercury after a sample is collected) and then roceeding as described by Peters and Van Slyke (1932) for introducing gas nd liquids into the chamber of the manometric apparatus. It is important o fill the bore of the top tap of the sampling tube with mercury before comnencing to transfer the sample of gas to the Van Slyke apparatus.

RESULTS OBTAINED WITH THE TWO FORMS OF APPARATUS

The type of data which have been obtained by the use of the two forms of pparatus is presented below. In a later paper extended data, obtained with kin-coated apples, will be presented and discussed in relation to the effect f skin-coatings on gaseous condition within the fruit. The skin-coatings were pplied to the apples (Cox's Orange Pippin) by means of a paint-spray gun sing compressed air.

Results obtained with Apparatus I

Comprehensive data illustrating the use of Apparatus I in a routine survey of the internal atmosphere of bulk samples of stored, coated fruit has been given in a report of a storage trial of 40 tons of Cox's Orange Pippin apples in a commercial store (Hulme, 1949).

The apparatus has proved valuable in making a rapid survey of the immediate effect of the application of skin-coatings of various compositions on the I.A. of apples.

Typical results of the effect of the concentration of oil in a skin-coating emulsion on the I.A. of the fruit are shown in Fig. 6. In this emulsion the oil phase consisted of arachis oil and medicinal paraffin in equal proportions. 'Lorol NH' (the ammonium salt of lauryl alcohol sulphate) was used as stabilizing agent at a concentration of 1 per cent. by weight. The samples of gas were withdrawn from the fruit at such a speed that the sampling tube was filled (i.e. emptied of mercury) overnight (approximately 18 hours).

In Fig. 6—as in later figures—the point at which the fruit was sprayed with the skin-coating emulsion is indicated by a vertical arrow above the letter 'C'.

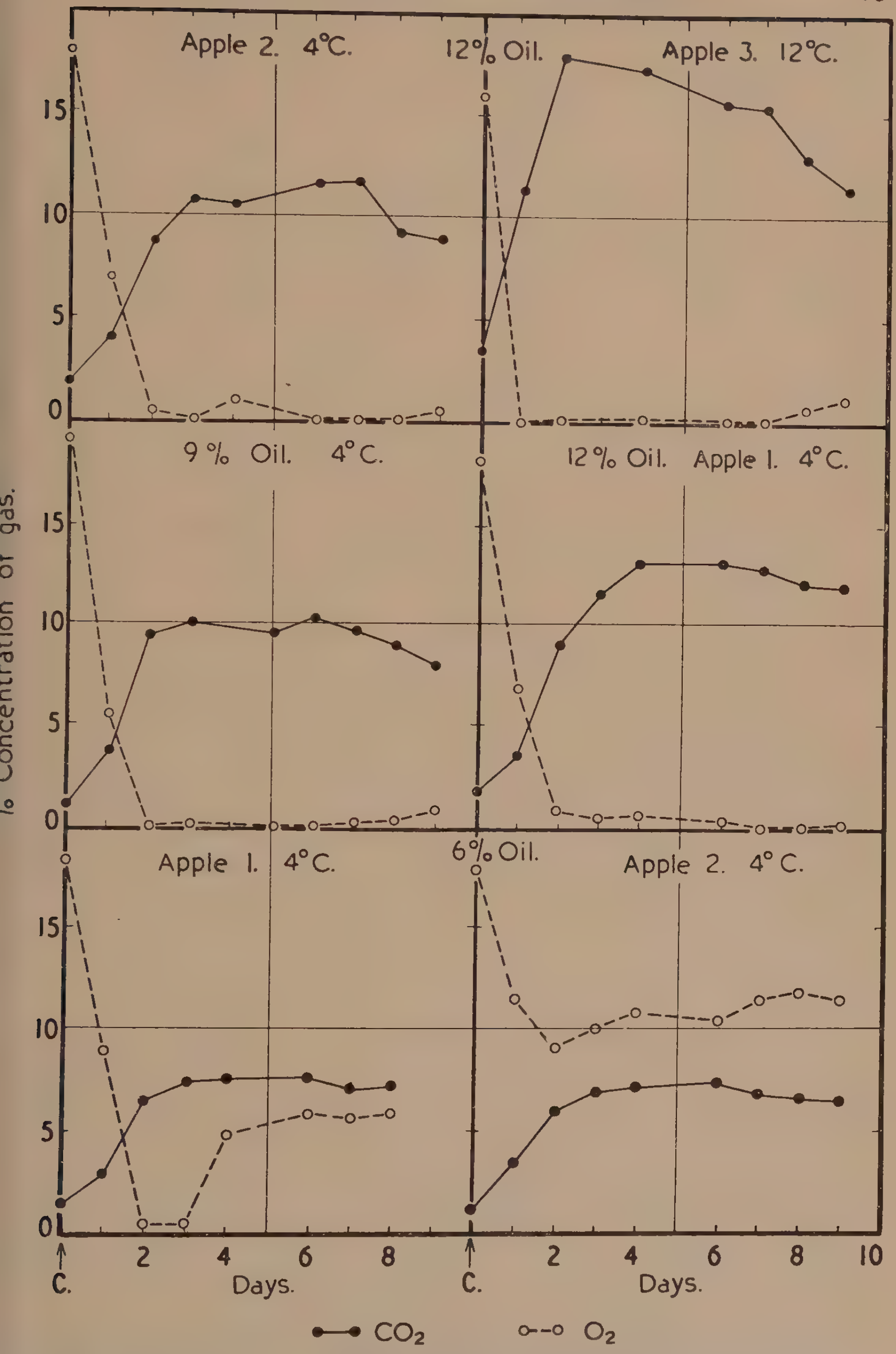
Besides demonstrating the decrease in O_2- and increase in CO_2-concentration within the fruit as the concentration of oil is increased, the effect of the temperature of the fruit during and after coating is also shown. Variation in response of apples from the same sample is also evident.

It appears probable that the variation in response to a given coating is due in part at least to the difficulty experienced in applying a coating of uniform thickness. Evidence in support of this suggestion is provided by the results shown in Fig. 7. At the point marked 'C 1' in the figure a 'light' coating (9 per cent. oil) was applied to the fruit which was given a second coating of the same emulsion at 'C 2'. As the number of coatings (and, therefore, the total thickness of the coating) is increased so the CO_2-concentration within the fruit rises and the O_2-concentration falls.

Results obtained with Apparatus II

1. *Internal pressures and internal atmospheres developed within a skin-coated apple.* The results described in this section were obtained at 15° C. with the chamber H (see Fig. 3) removed and the second manometer M 2 not in use. Tap T 4 of manometer M 1 was in the position connecting the right-hand limb of the manometer to air throughout the experiments.

The importance of avoiding the setting up of a negative pressure inside the fruit during the withdrawal of a sample is obvious: no appreciable negative pressure developed inside the fruit if samples of 1 ml. gas (the approximate volume of the sampling tubes) were withdrawn over periods greater than 1 hour. With an apple in which the composition of the I.A. had reached a steady state no appreciable difference in the composition of the I.A. was observed when samples were withdrawn over periods from 1 to 18 hours (see also Smith, 1947).



IG. 6. The internal atmosphere of apples coated with oil-water emulsions containing increas-
ng concentrations of oil. The point at which the coating was applied to the fruit is indicated by the letter 'c' at the foot of the graph.

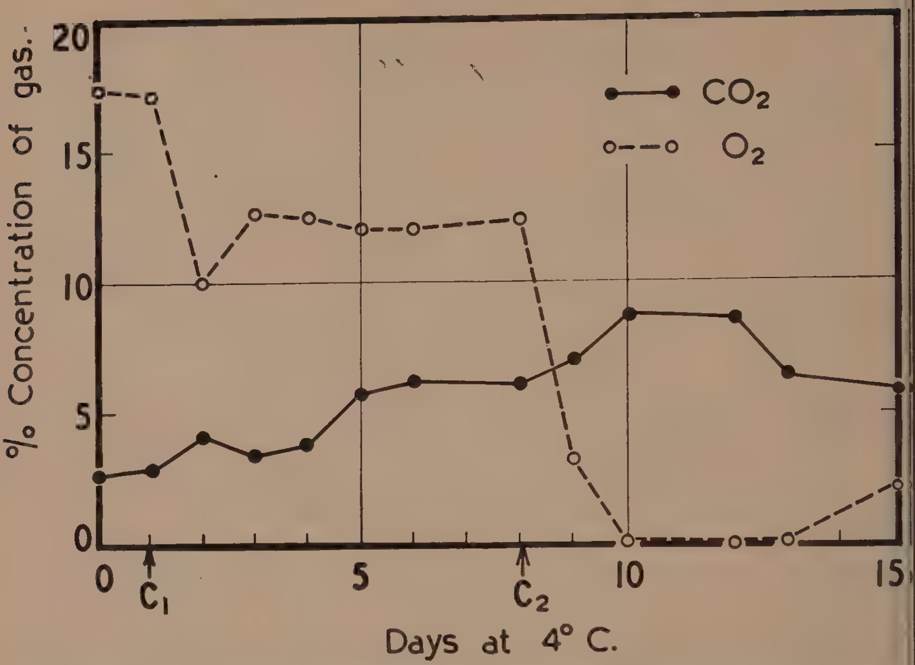


FIG. 7. The effect of two skin-coatings on the I.A. of an apple. 'c_1' and 'c_2' at the foot of the graph indicate the points at which the two coatings were applied.

The apple on the platform was either connected to manometer M 1 and then sprayed with a liquid and the change in pressure within the apple followed continuously for any length of time after spraying or, by suitable adjustment of the taps, a sample of gas was collected. This procedure may be alternated, but when the fruit is connected again to the manometer after a sample of gas has been removed it will take a little time for the I.P. to reach equilibrium (momentary), i.e. to recover from the withdrawal of the sample, especially when the withdrawal time is short and when pressures in the apple are close to atmospheric pressure. These 'adjustment' phases are shown in Fig. 8. In this figure (curve 1) the lines representing observed readings of the manometer are shown as continuous lines. The discontinuous line represents the probable course of the I.P. within the apple during the period when samples of gas were being withdrawn from the fruit (indicated by brackets and 'I.A.'). That this 'reconstructed' line approximates closely to the real drift in the I.P. is suggested by curve 2, which represents the observed I.P. after the application of a similar coating to a similar apple at a later date. Curve 2 is based on readings of the manometer taken at 10-minute intervals over the whole 400 minutes. It will be noted that the pressure changes during adjustment are too small to affect seriously the concentration of CO_2 in the I.A.

Fig. 9 shows the I.P. of two apples following the application of an oil-emulsion coating and the composition of the I.A. of the fruit at the mid-point of the period (1–2 hours) during which a sample of gas was collected for

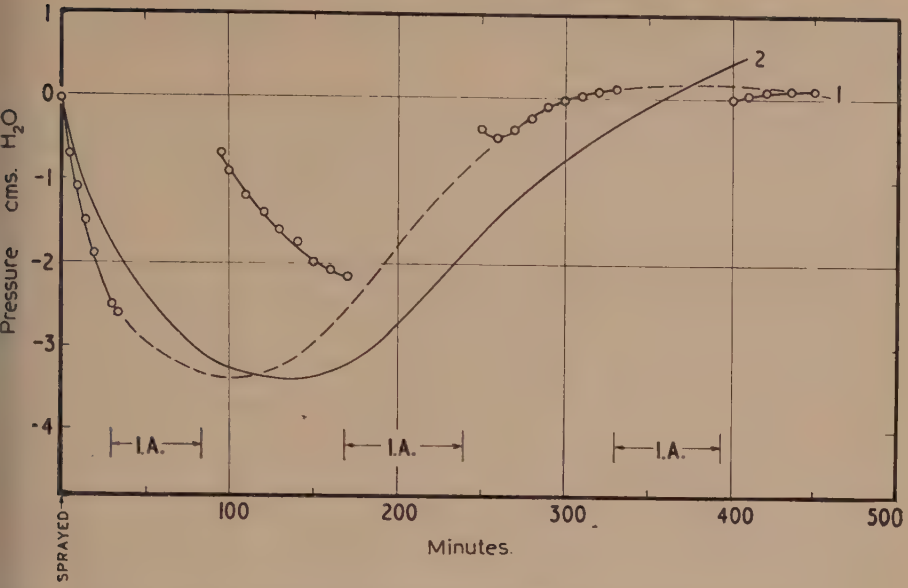


FIG. 8. The I.P. (at 15° C.) of an apple following the application of a skin-coating. (See text.)

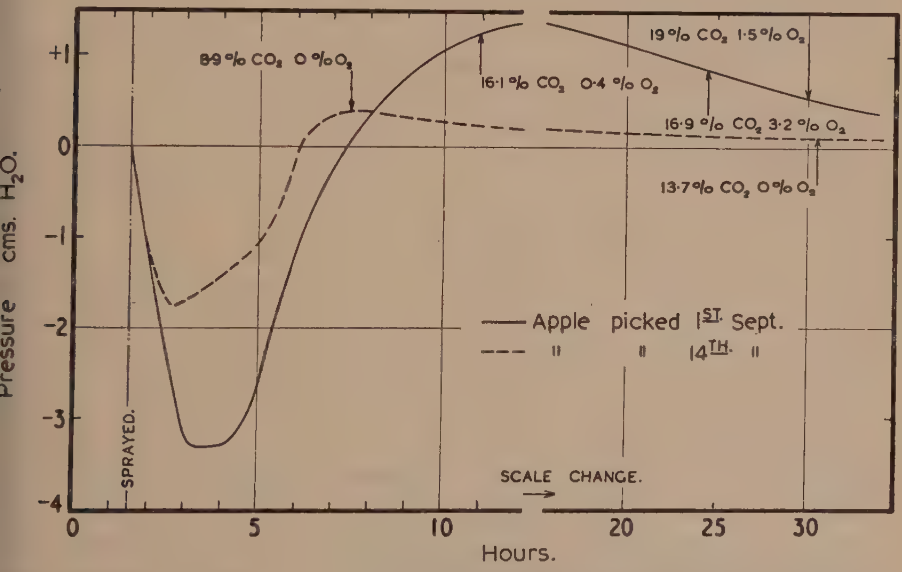


FIG. 9. The I.A. and I.P. (at 15° C.) of an apple following the application of a skin-coating. (See text.)

nalysis. Spraying results in a rapid rise in the concentration of CO_2 (the I.A. f both apples before spraying was normal, i.e. approximately 5 per cent. CO_2 nd 15 per cent. O_2) and a relatively rapid fall to a negative internal pressure ollowed by a rise to a positive pressure. The higher the ultimate concentration of CO_2 the more rapid is the fall in pressure and the higher is the subsequent maximum positive pressure. The implications of these results will be dicussed in a later paper.

Before proceeding farther there is one other factor to be considered. When an aqueous spray is applied to the surface of an apple the water will immediately commence to evaporate at a rate dependent on the relative humidity of the surrounding atmosphere. This evaporation will cool the surface of the fruit and this cooling effect might be sufficient to cause an appreciable temporary negative pressure inside the fruit. This might account for a considerable portion of the negative pressure observed inside an apple immediately following coating. To test this possibility, thermocouples (see Fig. 5) were inserted at various depths inside the apple and the temperatures as well as the I.P. were measured immediately following the spraying of the apple with a 1 per cent. aqueous solution of a wetting agent. The results obtained are shown in Fig. 10. It will be seen that, while there is a considerable reduction which may persist for 1–2 hours in the temperature of the apple near its surface, the lowering of the temperature of the apple as a whole is unlikely to exceed 0·5° C. The lower temperature at 20 mm. as compared with 10 mm. has no special significance since the measurements at 4 and 10 mm. (as well as the pressure measurements) were made on one apple and those at 1 and 20 mm. on another apple.

If it is assumed that the fruit is initially at atmospheric pressure (760 mm.), that on coating it becomes quite impervious to gases, and that the volume of its internal gas-volume does not change as a result of coating, then, according to Boyle's and Charles's laws, a fall in temperature from 15° C. to 14·5° C. will itself bring about a reduction in the I.P. of the fruit of 1·38 mm. of mercury or 1·87 cm. of water. This is about 4 times the fall in pressure actually observed in the apple (Fig. 10, interrupted line).

The fall in pressure inside the fruit following spraying with water containing low concentrations of various wetting agents varies considerably, the highest value obtained at 15° C. being 1 cm. of water. This variation is probably due to variation in spraying time and rate of evaporation of the water.

Although a continuous water-film round the fruit should render it impermeable to air (see later), such a film will have a transitory existence owing to the evaporation of the water. That this evaporation at 15° C. is rapid is obvious from the form of the curves illustrating the change of temperature of the surface of the apple. The breaking of the water film on evaporation will allow entry of air and the negative pressure inside the fruit will be dissipated. The approximate correspondence of the form of the pressure and temperature curves supports this argument.

It may be concluded, therefore, that while a fraction of the fall in pressure observed when an apple is coated may be due to a temporary cooling of the fruit, this fraction is only a small one in relation to the total negative pressure which develops as a result of spraying the fruit with an oil emulsion. In any case the cooling effect is transitory (50–60 minutes at most), whereas the

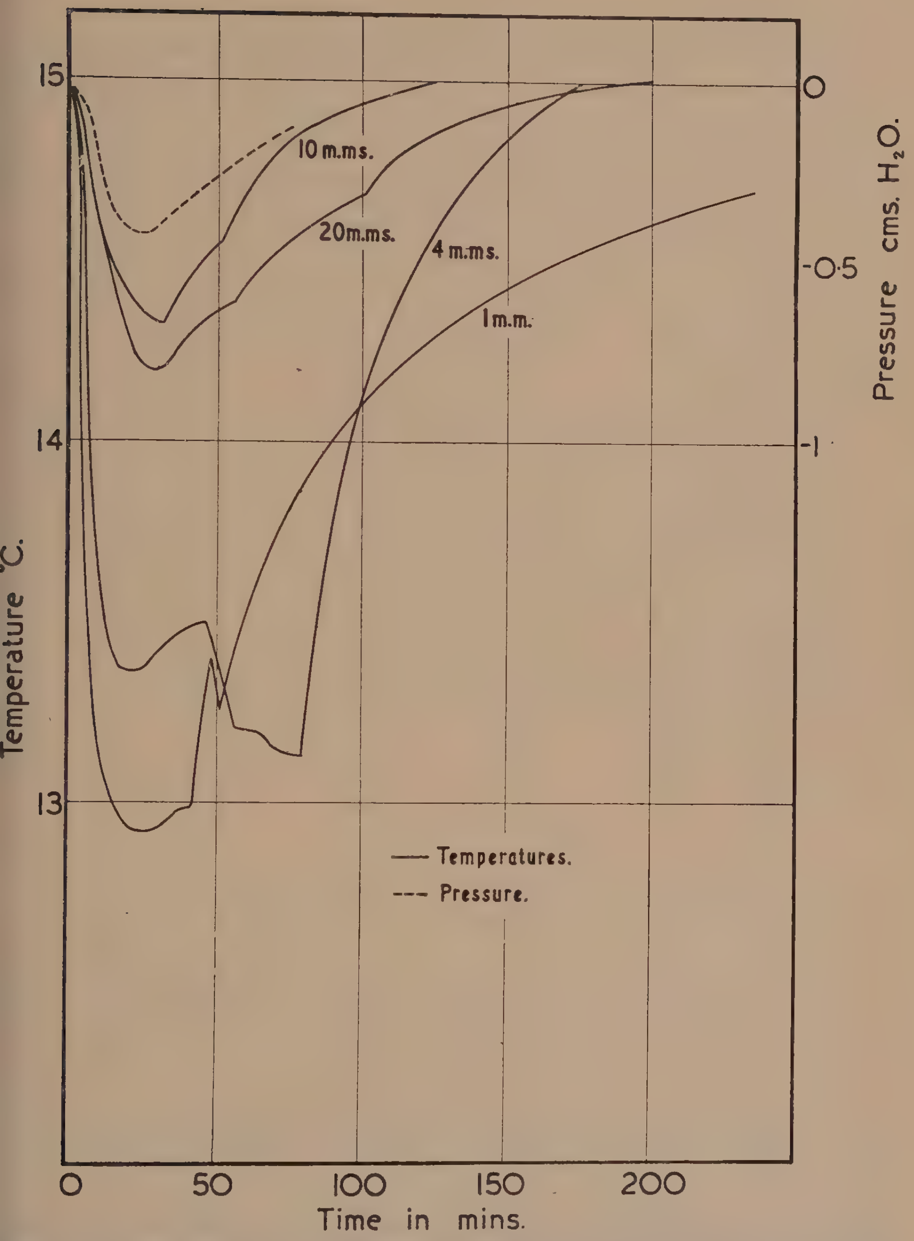


FIG. 10. The temperature at various distances below the surface of an apple after spraying the surface with water containing a wetting agent (two apples used, both of diameter 5·5 cm.). The pressure developed in one of the apples is also shown. (See text.)

negative pressures observed in oil-emulsion-coated fruit persist for several hours (see Fig. 9).

2. *The relative permeability of an uncoated and coated apple.* As mentioned earlier, the apparatus (II) also makes possible a study of the permeability to gas (air) of an apple before and after coating. The apple chamber is put under a slight negative pressure (the extent of this pressure may be measured by means of manometer 2) by sucking CO_2-free air through it against a resistance.

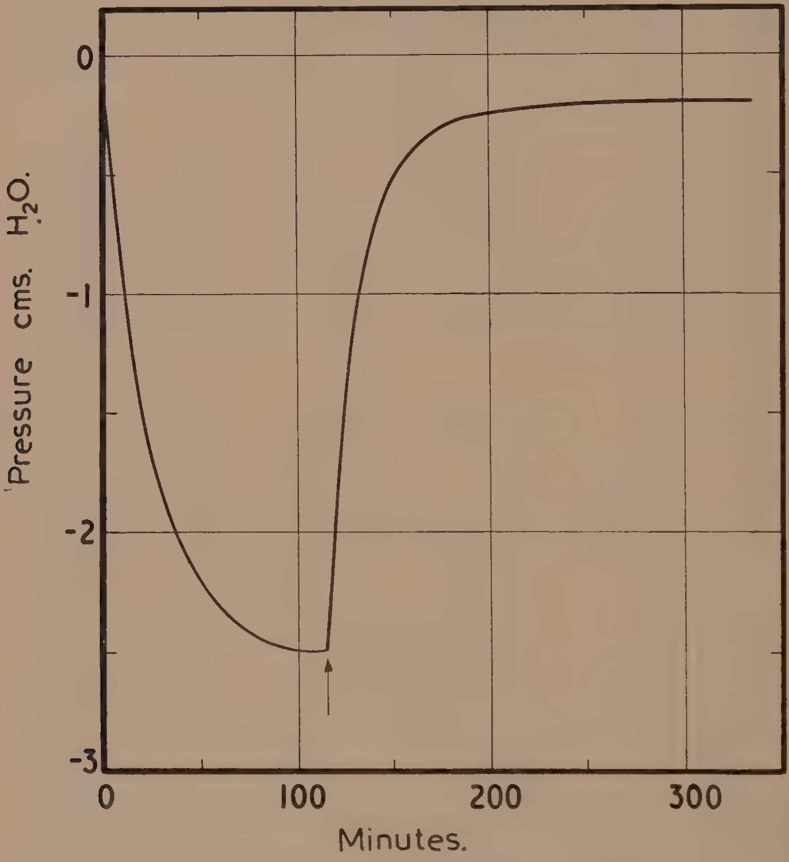


FIG. 11. 'Permeability' curve for an uncoated apple. (See text.)

In the present case the usual train of Pettenkofer tube, drier, flowmeter, needle valve, and soda-lime tube used in the measurement of CO_2 output served as a suitable resistance. If there is no apple in position on the platform P (Fig. 3) the meniscus of manometer M 1 will rise *immediately* to a position equivalent to that of manometer M 2. With a fruit in position, however, the manometer rises only slowly in the manner shown in Fig. 11. When the rise reaches an asymptotic value, tap T 4 is turned so that the right limb of manometer M 1 is subjected to the same negative pressure as the outside of the apple (point marked with an arrow in the figure). If the apple provided no resistance to the flow of gases, the meniscus of manometer M 1 should ultimately return to zero. Actually the fall in the meniscus (rise in pressure) reaches an asymptotic value appreciably below zero (Fig. 11). The shape of these 'fall and return' curves must be a function, primarily, of the resistance to gases of the tissue of the epidermis and cortex. All uncoated Cox's Orange Pippin apples so far tested give the same form of curve.[1] A skin-coated apple, however, gives a different permeability curve and differences are noted with different types of coating. For example, 24 hours after applying a coating composed of an oil-water emulsion containing 12 per cent. oil (arachis oil and medicinal paraffin in equal portions) and 1 per cent. of a wetting agent to the surface of the apple giving the 'permeability curve' illustrated in Fig. 11, an entirely different form of curve was obtained. This curve is shown in the upper half of Fig. 12 (continuous line). Another 24 hours later a curve similar in form but with certain differences (interrupted line) was obtained. Again the permeability curve obtained for a similar apple coated with a 5 per cent. aqueous solution of commercial sample of an alkyl cellulose (containing the usual wetting agent) was similar in form to that of an uncoated fruit but with a much extended time scale—Fig. 12, lower half. Presumably these differences in the permeability curves of untreated and treated fruit are due to the altered resistance of the 'skin-area' to gases (strictly, air) brought about by the various coatings. From the two results given in Fig. 12 it would appear that an oil-emulsion film has quite a different effect from an alkyl-cellulose film. Measurement of the I.A. of fruits coated with different preparations confirms this supposition.

DISCUSSION

To consider first the internal atmosphere of fruit coated with an oil-water emulsion: It will be noted that in all cases where apples have been coated with an oil-emulsion the combined concentration of CO_2 and O_2 in the I.A. is less than 21 per cent., the concentration present in non-senescent, uncoated fruits. Whatever the cause—and this will be discussed in a later paper—this deficit in CO_2+O_2 is important in relation to the storage of fruit. The well-known principle of 'scrubbed'[2] gas storage as applied to varieties such as the Cox's Orange Pippin depends for its success on such a deficit. The 'optimal' gas-storage conditions for this variety are 4–6 per cent. CO_2 combined with 2·5–3·5 per cent. O_2 (Kidd and West, 1936). Almost as good storage results can be obtained with a composition of 5 per cent. CO_2 and 5 per cent. oxygen. With such a relatively crude method for the regulation of gaseous exchange between the apple and its surrounding atmosphere as a skin-coating, too low a concentration of O_2 must not be aimed at since concentrations in the atmosphere surrounding the fruit in the region of 3 per cent. or lower are known to produce abnormal amounts of alcohol within the fruit (Thomas and Fidler, 1933). The concentration of O_2 inside the fruit will, owing to its consumption in respiration, be somewhat lower than that of the surrounding atmosphere. The composition of the atmosphere of fruit surrounded by a mixture of 5 per cent. CO_2 and 5 per cent. O_2 (and 90 per cent. N_2) can be measured in apparatus II by passing a gas mixture of this composition through the apple chamber and, from what has already been said, it should be possible to obtain a similar composition in a fruit by the application of a skin-coating. The results shown in Fig. 13 illustrate that this is possible. In practice it has been

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[1] The time taken to reach equilibrium increases as the fruit becomes more senescent.

[2] 'Scrubbed' gas storage means storage in mixtures of CO_2 and O_2 controlled by a 'scrubber' apparatus which removes a portion of the CO_2 produced in the store by the fruit itself. This enables CO_2 and O_2 mixtures of less than a total of 21 per cent. to be obtained.

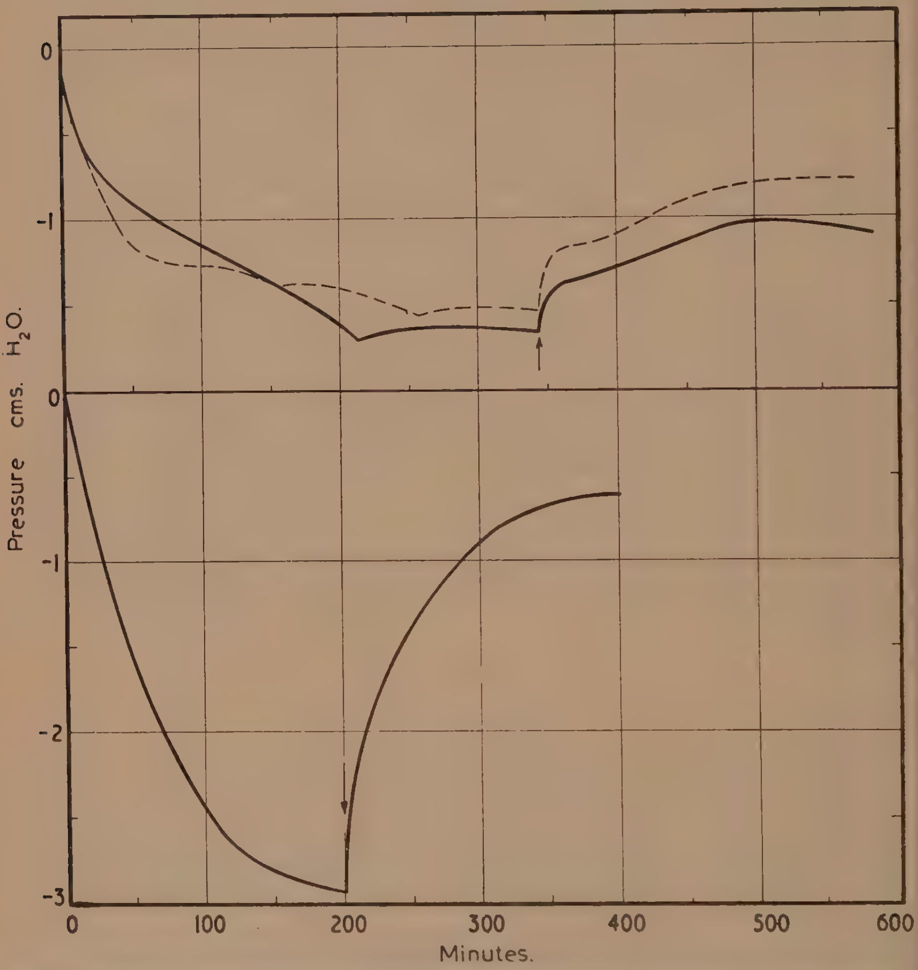

FIG. 12. 'Permeability' curves for an apple coated with an oil-emulsion (upper curve) and with 5 per cent. alkyl-cellulose (lower curve).

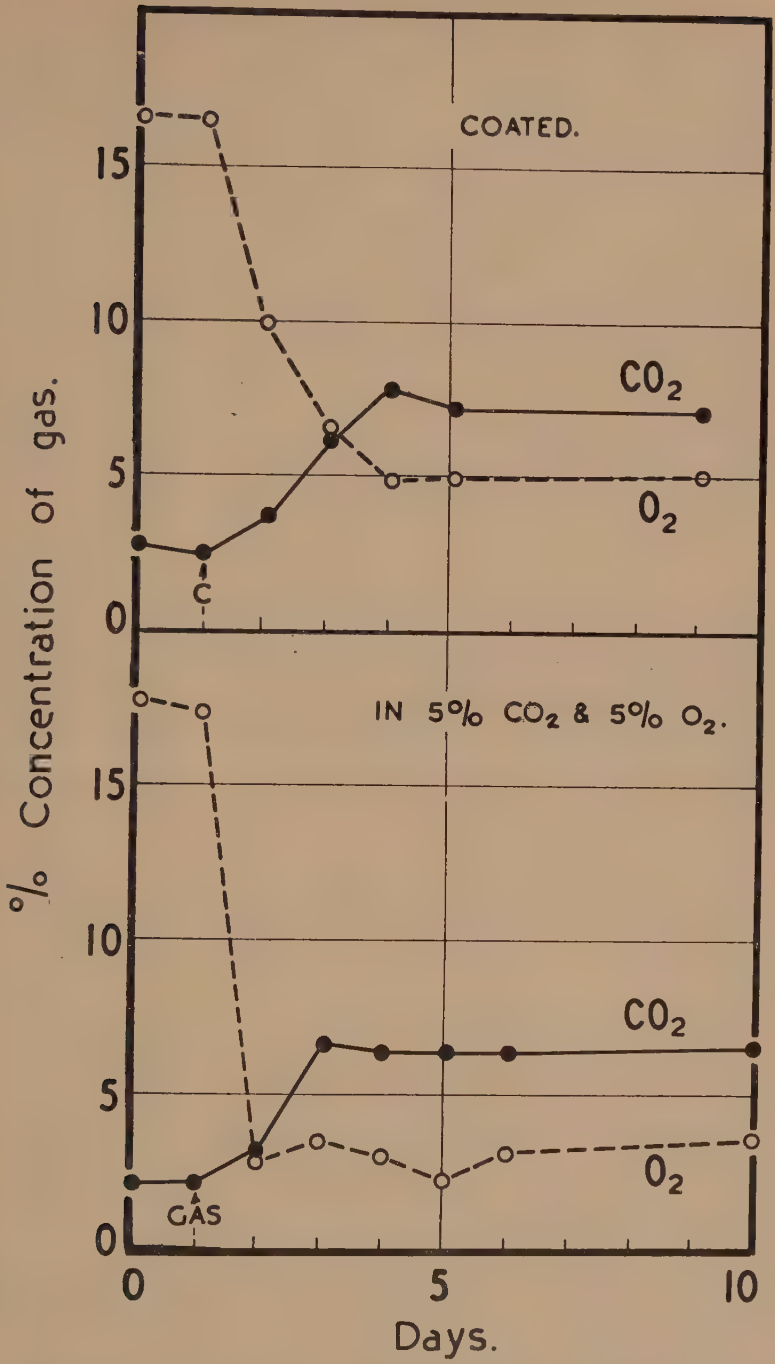

FIG. 13. A comparison of the I.A. of a skin-coated apple and one surrounded by an atmosphere of 5% CO_2 and 5% O_2.

found that only a small percentage of a population of apples treated with the same coating will have an initial atmosphere of the 'ideal' composition.

It appears probable that the 'scrubbed' gas-storage effect produced in fruits by a skin-coating composed of an oil emulsion is due to the different solubilities of CO_2 and O_2 in oil. Presumably, when the coating is first applied the oil-in-water film while being fairly permeable to CO_2 is almost impermeable to O_2 (O_2 is practically insoluble in water). As the water evaporates and leaves behind a film composed entirely of oil, O_2 is enabled to enter to a limited extent owing to its appreciable solubility[1] in oil.

It has been demonstrated that the internal pressure within a coated fruit varies with the composition of the internal atmosphere and that the 'permeability' of the skin of an apple is profoundly affected by the application of a coating. A study of these factors by means of the two apparatuses described should lead to the development of a skin-coating—not necessarily a 'straight' oil-water emulsion—which, by employing the principles of gas-storage, would add to the commercial life of apples in cold storage.

SUMMARY

1. Two forms of apparatus are described, one for the routine analysis of the internal atmosphere of an apple fruit and another for the measurement of (i) carbon dioxide output, (ii) internal gas concentrations, (iii) atmospheric pressure within the fruit, (iv) permeability of the fruit to gases.

2. The use of these two apparatuses to follow changes within the fruit as a result of the application of a skin-coating is described and the results obtained are discussed.

3. It is shown that the fall in pressure which occurs as a result of the cooling of the fruit due to evaporation of the applied coating can only form a small fraction of the total fall in pressure observed as a result of the application of the coating.

4. It is shown that coating the fruit with an oil-water emulsion causes a deficit in the combined concentration of CO_2 and O_2 within the fruit below the 21 per cent. observed in similar uncoated fruit.

5. An explanation of this phenomenon is advanced, and it is suggested that the modification of the internal atmosphere brought about by the skin-coating, if it can be suitably controlled, will prove valuable in increasing the cold-storage life of apples.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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[1] The Bunsen coefficients for the solubility of CO_2 and O_2 in oil—the only oil for which data appears to be available is 'transformer oil'—at 25° C. are, respectively, 0·99 and 0·156.

ACKNOWLEDGEMENTS

The non-glass parts of apparatus I were designed and constructed by the late Mr. W. H. Piper and of apparatus II by Mr. F. G. E. Forsyth. Many of the determinations of internal atmosphere were made by Mr. W. Arthington.

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Studies in Stomatal Behaviour

III. THE SENSITIVITY OF STOMATA TO MECHANICAL SHOCK

Part 2. True Shock-phenomena and their Implications

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SUMMARY

If *Pelargonium* stomata, wide open under a porometer-cup, are subjected to a mechanical shock and then darkened, subsequent closure is markedly accelerated. The effect persists for at least 18 hours and, provided a relatively lengthy dark period quickly follows the shock, is unaffected by subsequent light periods. If, however, the shock is followed by a light period of about 2 hours, subsequent closure is decelerated, and this deceleration similarly persists through subsequent dark periods. The effects can largely be explained on the basis of shock-induced respiratory changes, if it is postulated that photosynthesis inhibits respiration in chlorophyllous tissue. The effect of the shock is not instantaneous, the new pattern of behaviour not being discernible in either case until the stimulated tissue has been exposed to light or darkness for perhaps an hour. It appears that in occasional sensitive leaves such phenomena may be induced by the slight shock involved in fitting a porometer-cup, and the conditions of light or darkness to which the cup is subjected immediately after fixing may then influence its subsequent behaviour.

INTRODUCTION

In the first part of this communication (Williams, 1949) the author showed that the apparent shock-closure, or 'Knight effect', observed on fixing a porometer-cup is a misinterpretation; but the stomata of *Pelargonium* do nevertheless show a small genuine shock-effect, manifest as a slight closing tendency on fixing the porometer-cup, and possibly attributable to a shock-induced respiratory increase. Since Audus (1935, 1939) has shown that such increases are remarkably persistent, it appeared possible that the extent to which the leaf had been handled during the attachment of the cup might be reflected in the subsequent stomatal behaviour; and this communication is a report on the unexpected results of a brief investigation into this possibility.

All experiments have been carried out on attached leaves of *Pelargonium zonale*, var. 'Paul Crampel', with the exception of two replicates of Expt. 8, in which the variety 'King of Denmark' was used. This variety, with its relatively flat smooth leaves, appears superficially more suitable for stomatal work than the familiar 'Paul Crampel'; but in the writer's experience its stomatal behaviour is sluggish and more unpredictable than is usual in *Pelargonium*, and its use has therefore been discontinued. The technique was

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that of the previous communication and, as before, in the graphs illustrating this paper manometer pressure has been taken as a measure of stomatal resistance, increasing pressure (to a theoretical maximum of $P = 12$ cm. dekalin) representing stomatal closure.

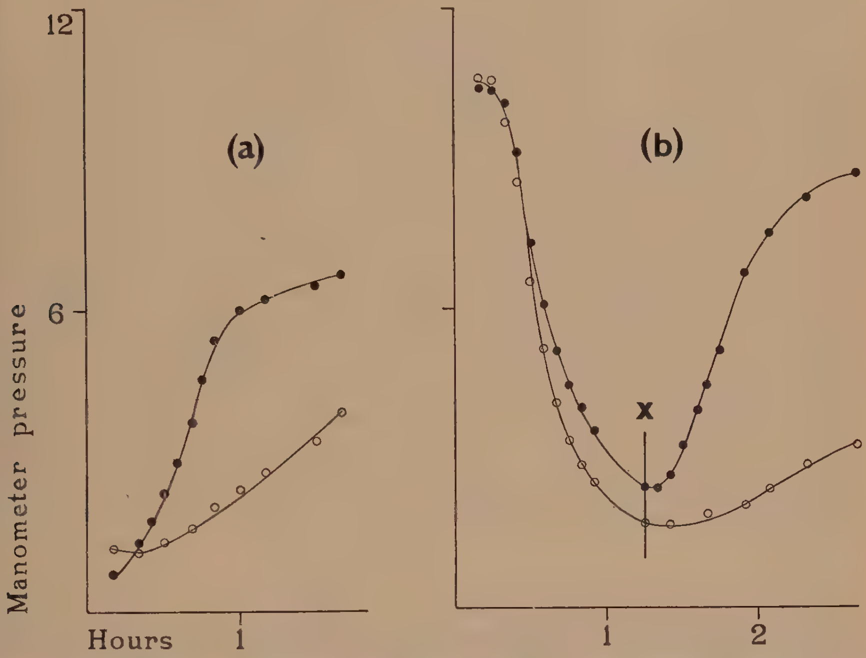

FIG. 1. Shock followed by darkness. Open circles, controls; closed circles, shocked. See text, Expt. 1.

I. ARTIFICIALLY INDUCED SHOCK PHENOMENA

1. *Shock followed by darkness*

Experiment 1. Two porometer-cups were attached to the same leaf, which was left in the light until the subsequent opening was substantially complete (about 2 hours). The glass plate covering one cup was then gently removed, the leaf over the cup subjected to a sharp pressure from the forefinger, and the plate replaced; both cups were then darkened by placing a halfpenny over each. The result is shown in Fig. 1 *a*. It is clear that the stomata in the 'shocked' region are showing an increased rate of closure compared with the controls. The cups were darkened overnight and illuminated the following morning; the result is shown in Fig. 1 *b*, from the beginning of the record to the point *x*, when both cups were again darkened. It will be observed that the effect has persisted.

This experiment has been repeated a large number of times and, although the degree and persistence of the effect vary considerably, the general principle is regarded as established. Now if, as seems clear from the work of

Heath (1948, 1950), stomatal closure is largely brought about by the accumulation of respiratory CO_2, this result is precisely what would be expected. Audus (1939) has shown that mechanical shock increases the respiration of a *Pelargonium* leaf to rather more than $1\frac{1}{2}$ times its normal value, and that the respiration may remain higher than normal for several days. No significant effect on the opening curves is to be expected; for the light-intensity (a few inches away from a 100-watt lamp) is believed to be well above the compensation-point for *Pelargonium*, and the photosynthetic process will no doubt be able to dispose of the increased CO_2 supply as rapidly as it will that from the normal level of respiration. Moreover, if we postulate that light largely inhibits respiration in chlorophyllous tissues, even this problem does not arise; this possibility is discussed below. However, when the leaf is darkened, the conditions in the two areas—the normal and the 'shocked'—differ: for respiratory CO_2 will accumulate faster in the 'shocked' region, and a corresponding increase in the rate of stomatal closure will follow automatically.

A possible alternative explanation must be mentioned: it is conceivable that the pressure over the cup has spread the luting-wax ring, thus decreasing the effective area of the porometer-cup. Inspection of the leaf at the close of a typical experiment failed to support this suggestion, but a better test would clearly be to affix, as gently as possible, two porometer-cups of different areas to the same leaf, and to observe the response-curves obtained. (I am indebted to Dr. O. V. S. Heath for pointing out the desirability of this experiment.)

Experiment 2, therefore, was carried out in this manner. Two cups were prepared with glycerol-gelatin rings of normal external diameter but differing internal diameters (5 and 12 mm.), and these two cups were attached to the same leaf. The behaviour of the stomata thus studied is shown in Fig. 2: first, the usual preliminary opening, then (at x) closure due to darkening, and finally (at y) reopening on illumination. It is at once clear that the relationship between the two curves differs from that shown in Fig. 1. First, the opening curve for the larger cup lies consistently below that for the smaller, a phenomenon never exhibited in any of the replicates of Expt. 1. Secondly, although the smaller cup shows a slightly increased rate of closure, the two rates of closure increase together and slacken off together; whereas in Fig. 1 the 'shocked' curve rises first and slackens off quickly, so that it is approaching equilibrium while the control curve is still rising. We may therefore conclude that the effect under discussion is not due to unintentional spreading of the wax ring.

2. *Shock followed by light*

Experiment 3. The records of Expt. 1 show that the shock-effect can persist for at least 18 hours; and it was thus natural to suppose that the effect would be manifest if the cups received their first dark period at any time up to at least 18 hours after fixing. An experiment was therefore carried out in which two cups were attached and the stomata allowed to open as before, one cup

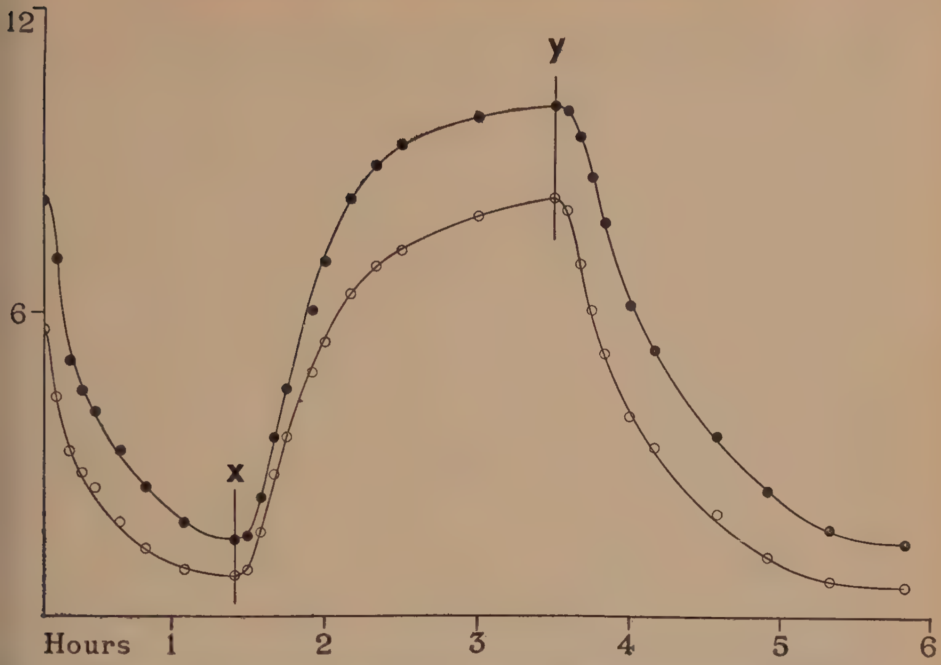

FIG. 2. Variation in size of cup. Open circles, large cup; closed circles, small cup. See text, Expt. 2.

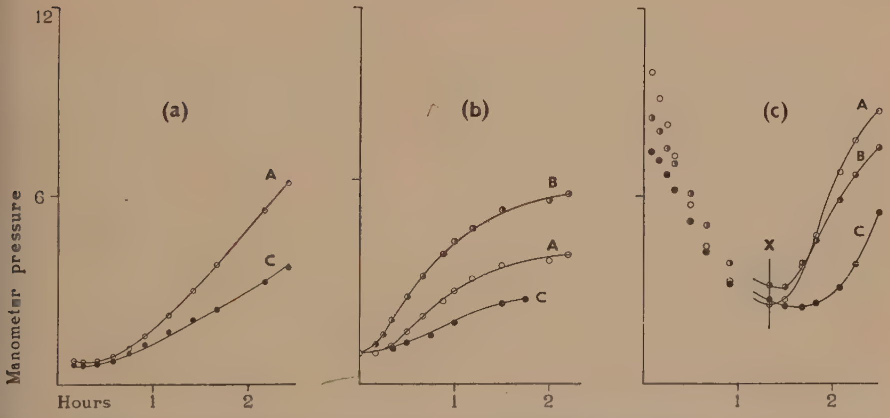

FIG. 3. Shock followed by light. *A*, controls; *B*, shock followed by darkness; *C*, shock followed by light. See text, Expts. 3, 4, and 5.

then receiving a shock; but, instead of darkening the cups immediately, they remained fully illuminated for 2 hours before being darkened. The result is shown in Fig. 3 *a*, from which it will be seen that the 'shocked' area is now showing a *decreased* rate of stomatal closure. This disconcerting result was naturally at first ascribed to some aberration of the leaf concerned; but further investigation seemed desirable.

Experiment 4. Three cups were attached to the same leaf, and the stomata under all three allowed to open in the light in the usual manner; this opening period is not shown in the records (Fig. 3 *b*). Cup *A* was a control, darkened immediately without mechanical interference; cup *B* received a shock and was darkened immediately as in Expt. 1; cup *C* received a shock, but, as in Expt. 3, was left fully illuminated for 2 hours, then darkened. The closing curve of cup *C*, in fact displaced from those of *A* and *B* by 2 hours, has for ease of comparison been plotted in Fig. 3 *b* as from the same origin. Cup *B* exhibits the usual accelerated closure, showing that the leaf was not abnormal in this respect; but cup *C* again shows a *decreased* rate of closure.

Experiment 5. Since in Expt. 4 cup *C* was darkened 2 hours later than the other cups (at 11.45 hours G.M.T.), it could be argued that the difference in response was attributable to diurnal rhythm, which is known to occur in *Pelargonium* (*vide* Gregory and Pearse, 1937, and especially Heath, 1949); Expt. 5 was designed to exclude this possibility. Three cups (*A*, *B*, and *C*) were attached to the same leaf and received treatments similar to those of Expt. 4; but no records were taken the same day, the cups remaining in darkness overnight. The following morning the cups were illuminated: the result is shown in Fig. 3 *c*, in which the entangled opening curves have not been drawn. At *x* the cups were darkened, and, although the acceleration that would be expected of *B* is not shown, *C* again exhibits retarded closure. Two further experiments of this type were carried out with similar results; moreover, it was observed in each case that, compared with those of *A* and *B*, the stomata of cup *C* closed to a lesser extent in the darkness overnight.

Experiment 6. Since a finger and not a transparent instrument was used for stimulation, the leaf is technically in darkness at the instant of receiving the shock; but it was considered desirable to repeat this type of experiment with the whole leaf darkened during stimulation. Three cups, *A*, *B*, and *C*, were attached to a leaf and the stomata allowed to open as usual. The light was then switched off (the experiment was carried out in a room dark except for the lamp over the leaf); after one minute *C* was given the usual shock; after a further minute the light was switched on; after a further 90 minutes' illumination *B* was given a shock, and all three cups were darkened. *A* was therefore a control, darkened with no mechanical disturbance; *B* was a normal 'shock followed by darkness' run; *C* was a 'shock followed by light' run, but with the leaf in complete darkness at the moment of stimulation. The results are shown in Fig. 4 *a*, from which it is clear that though the closure of *B* is less accelerated than is common, the closure of *C* is as expected retarded.

Experiment 7. In this, *A* and *C* were as in the previous experiment, except that *C* was shocked in the middle of an 8-minute dark period; the third cup, now labelled *D*, was a normal 'shock followed by light' run. The results, shown in Fig. 4 *b*, are as expected: both *C* and *D* show slightly decelerated closure compared with *A*.

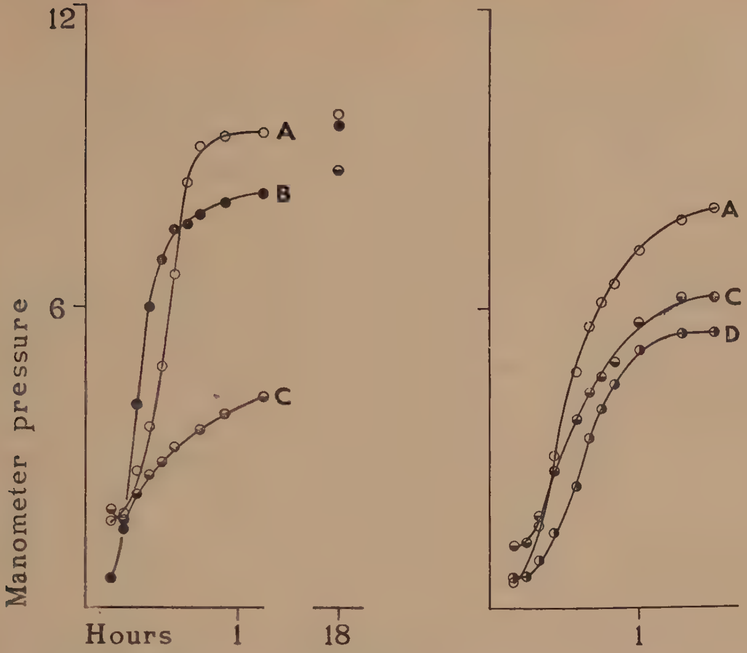

FIG. 4. Shock with whole leaf darkened. For explanation see text, Expts. 6 and 7.

3. *Discussion*

Unless by some mischance every one of the experiments in section 2 represents a fortuitous anomaly, the conclusion seems inescapable that whereas a shock followed immediately by darkening brings about a marked acceleration of stomatal closure, a similar shock followed by a period (in this case 1–2 hours) of light results in a corresponding retardation of closure on later darkening. If the respiratory hypothesis invoked in the description of Expt. 1 is maintained, shock followed by darkness (as, incidentally, was always the case in Audus's experiments) *increases* respiration: shock followed by light *depresses* it. There is no difficulty in accepting an increased respiration when darkness follows immediately after shock; but, since both shock (Audus, 1935, 1939) and light (Audus, 1947) are known to increase subsequent dark-respiration, it is difficult to conceive that an interaction between them would bring about its reduction.

However, evidence is accumulating from studies in the kinetics (Kok, 1948, 1949) and chemistry (Calvin, 1949; Benson and Calvin, 1950) of photosynthesis that light inhibits aerobic respiration in such parts of the cytoplasm as are within the sphere of influence of the chloroplasts. Now Audus (1940) has shown that the shock-induced respiratory increase resides not in the presumed glycolytic stages of respiration, but in the aerobic chain itself. Stimulation has no effect on anaerobic respiration, presumably because the aerobic process, on which alone it acts, is not in operation; it seems reasonable to postulate that stimulation has no effect on respiration in light for the same reason.

Two points of interest arise. First, this hypothesis requires that shock followed by light should have no effect on subsequent dark-respiration, not that it should depress it. The deceleration of closure under these conditions is admittedly less than the acceleration exhibited when darkness quickly follows the shock; but it seems to be real, for it has been observed in all experiments. The writer offers no explanation.

Secondly, we gain some information on the shock-effect itself. It is clearly not instantaneous; Expts. 6 and 7 show that even though the leaf is stimulated in darkness (when the aerobic process is no doubt in operation) and left in darkness for several minutes after the shock, the following 90-minute period still causes retardation. On the other hand, if the leaf is darkened continuously for some hours after the shock, the resulting acceleration is not suppressed by any subsequent light periods. The shock apparently sets up some form of secondary disturbance which persists for 1 to 2 hours; this disturbance so affects the aerobic process as to cause it to run at a changed dynamic equilibrium; but an appreciable period of time (1–2 hours) is required to set up the new equilibrium which, once set up, only slowly (in two or three days) reverts to the normal level. There is perhaps a parallel to be drawn here with Audus's observations (1947) that exposure to light increases subsequent dark-respiration to a degree dependent on the period of illumination, 'building up with time to an asymptotic value', and that the original level is regained only after 2–3 days. The nature of the postulated disturbance hardly merits discussion at this stage; it is perhaps worth mention that it is unlikely to be electrical since, unlike heat-shock, mechanical shock is not known to induce electrical responses in the leaves of *Pelargonium*.

Finally, it must be emphasized that the writer offers no *direct* evidence for changes in respiration. However, the postulated respiratory changes are at least plausible, and it is clear that, in any future work on the effect of mechanical shock on leaves, the possibility of an interaction between shock and light such as is recorded here should be borne in mind.

II. APPLICATION TO NORMAL TECHNIQUE

The existence of an interaction between mechanical shock and light such as is suggested above might entail certain disquieting consequences in normal stomatal technique. For the mere fixing of a porometer-cup is itself a mild mechanical shock; and it is conceivable that the stomata will differ in their behaviour according to whether the leaf has been illuminated or darkened after the cup was fixed. Since this has not normally been regarded as a point of technique sufficiently important to warrant standardization, it was felt desirable to carry out a brief investigation.

Experiment 8. Two cups were attached to the same leaf as gently as possible; one was immediately darkened, the other left illuminated for not less than 2

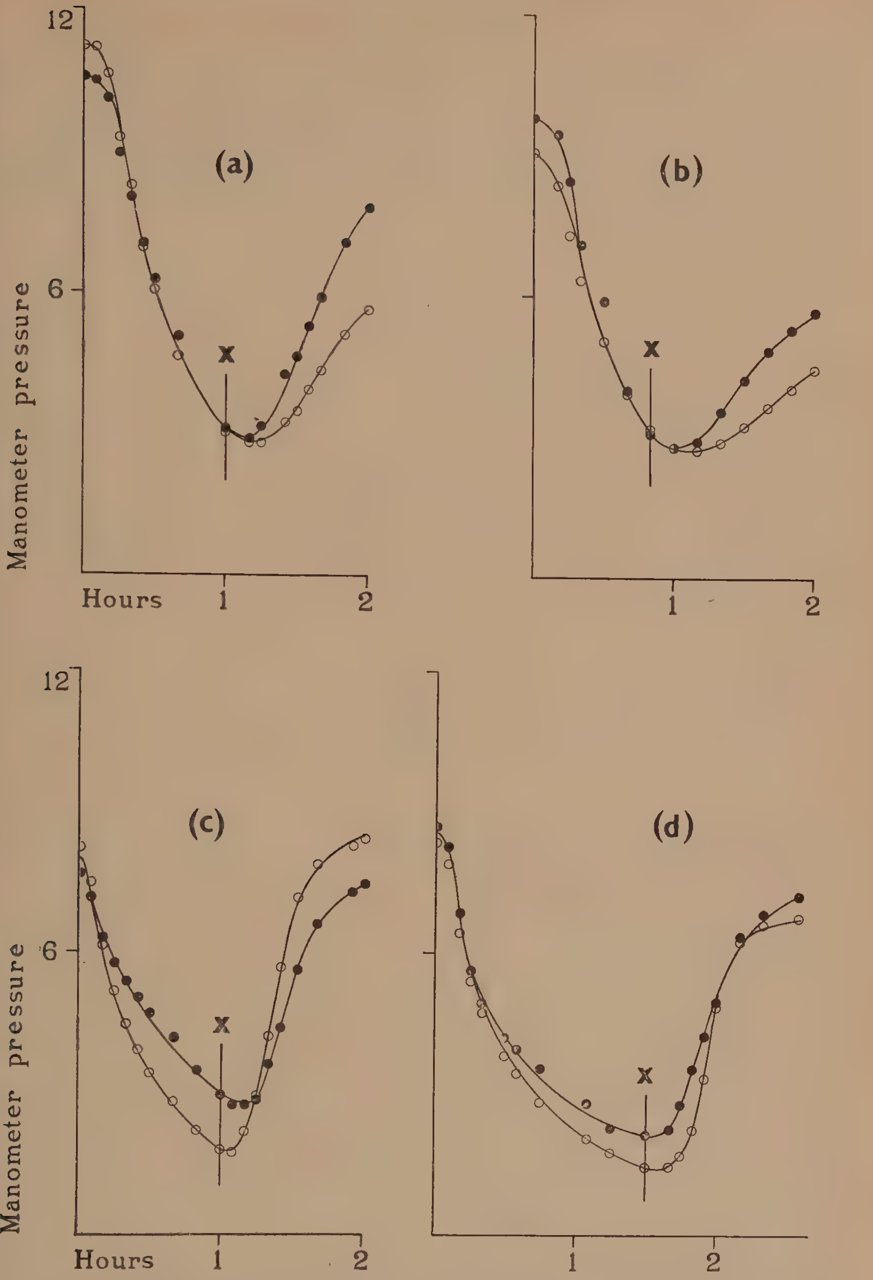

FIG. 5. Normal technique. Open circles, 'recovery' in light; closed circles, 'recovery' in darkness. See text, Expt. 8.

hours. Both were then darkened overnight. The following morning both cups were illuminated, then, after a suitable interval, darkened; four records so obained are shown in Fig. 5, in each of which *x* represents the time of darkening.

It will be clear that these results are inconclusive: the records (*a*) and (*b*) (both var. 'King of Denmark') might be considered to show a positive result, but, if so, (*c*) and (*d*) (both var. 'Paul Crampel') are positive in the reverse sense. However, six such experiments were carried out in all, the records of the remaining two being shown in Fig. 6. These are strikingly positive, and in the right direction: it would appear, then, that if the phenomenon is exhibited at all it is as expected.

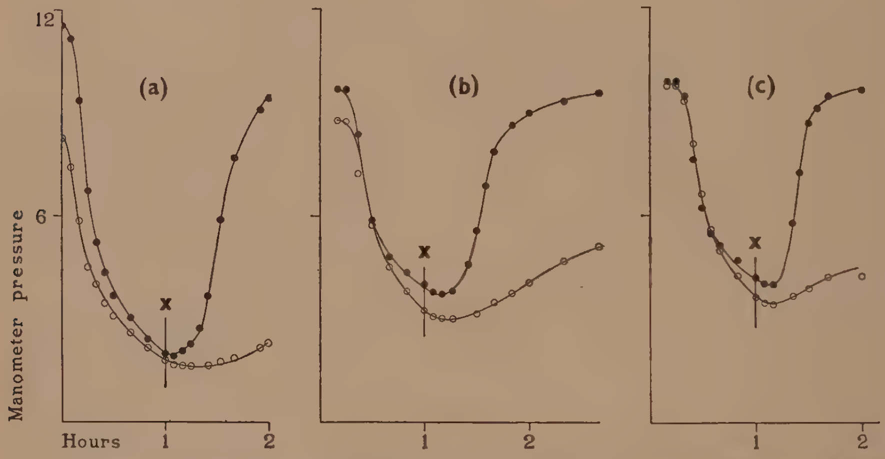

FIG. 6. Normal technique. (*a*) and (*b*) are replicate experiments; (*c*) is a further record of (*b*) taken the following day. See text, Expt. 8.

The shock attendant on the careful fixing of a cup is evidently too slight to induce the phenomenon consistently, and it was at first suspected that the cups which gave the striking results of Fig. 6 had inadvertently been fixed with undue violence. Experiments were therefore carried out in which one cup was fixed as gently as possible, and the other intentionally mishandled, both cups being allowed to 'recover' under the same conditions of light and darkness. The results, though suggesting a slightly increased sensitivity in the roughly handled cup, were insufficiently conclusive to justify quoting in detail, and certainly could not be invoked to explain differences of the order of those seen in Fig. 6. The writer can only suggest that this difference reflects merely a difference in inherent sensitivity of individual leaves, or possibly difference of age, since in *Pelargonium* leaf-size gives little indication of leaf age, and leaves for two-cup experiments are selected mainly for size.

Our only conclusion, therefore, must be that stomata under a porometer-cup, in circumstances at present largely obscure, differ in their subsequent behaviour according to the conditions of light or darkness to which they are subjected in the period immediately following the fixing of the cup; and that, until this effect is fully understood, it is wise to standardize this aspect of porometer technique. It would appear that, if there is to be any effect at all, increased sensivity is to be gained by allowing the leaf to 'recover' in darkness.

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The Effects of Temperature on the Durations of the Different Stages of Cell Division in the Root-tip

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SUMMARY

A new technique for determining the durations of the different phases of division is described.

It is shown that at 15° C. the mean durations of the several phases in the root-tip of the pea are as follows: interphase 23 hours; prophase 2 hours; metaphase 25 minutes; anaphase 5 minutes; telophase 22 minutes.

The effects of different temperatures have been determined, and it is shown that all stages are accelerated by an increase in temperature from 15° to 25° C., the ratios of the durations at the two temperatures being about 2·0 or slightly less.

It is suggested that since interphase is the longest stage of the division cycle, in terms of growth effects on this stage are the most significant.

INTRODUCTION

The observations that are described below represent a contribution to an elucidation of the factors that control the growth of the root. Brown and Rickless (1949) have described a method for the measurement of the over-all rate of cell division, but further analysis requires observations on the effects of various treatments on the separate phases of division. A method for doing this has been developed in the course of this investigation, which is primarily concerned with the effect of different temperatures on division in the tips of intact roots of seedling peas.

In an initial stage of this investigation an attempt was made to measure the durations of the several phases of division with the experimental material of Brown and Rickless. These workers used isolated tips of *Cucurbita*. It was found, however, that the meristem of *Cucurbita* does not stain readily with aceto-carmine, and this is an essential feature of the phase-timing technique. Further, the use of isolated tips has been abandoned since conditions in these may change rapidly with time. The use of seedling roots has the additional advantage that variability between these is less than it is with isolated roots.

We have investigated the durations of interphase, prophase, metaphase, anaphase, and telophase with four temperatures, 15°, 20°, 25°, and 30° C., with the roots in water and in the mineral salt solution used by Bonner and Addicott (1937) for culturing isolated roots of peas. As shown below, with the intact seedling roots the mineral nutrient has little effect in the conditions of the present series of experiments.

Journ. of Experimental Botany, Vol. II, No. 1.

METHODS AND TECHNIQUES

In a system such as that of the root, where divisions throughout the mass of meristematic cells are more or less random, the relative number of cells in any one stage of division at any time is proportional to the fraction of a total duration of a division cycle that is occupied by the duration of the particular stage. This consideration is the basis of the method developed here. An estimate of the over-all rate of division is obtained. At the same time the percentage numbers of cells in the different stages of division in the mass of meristematic cells is determined. From these two sets of data the duration of each phase is calculated by multiplying the total duration by the fractional value corresponding to the percentage number of cells in that phase. Thus, if the length of a cycle is 24 hours and the proportion of cells in a particular phase is 5 per cent., then the duration of that phase is $24 \times 0{\cdot}05$, or 1·2 hours.

Clearly the method is only applicable if divisions in the meristem are approximately random in the sense that the probability of any one cell entering the mitotic phase is the same throughout a succession of equal time-intervals throughout a 24-hour period. This condition necessarily implies that the behaviour of each cell is independent of that of other cells. Data showing that this condition is approximately fulfilled are presented below in a section on the limitations of the method. The presentation of these data, however, is preceded by descriptions (1) of the methods of culturing the seedlings, (2) of determining the over-all rate of division, (3) of determining the percentages of cells in the different phases in the mass of meristematic cells, (4) the general design of an experiment, and (5) the errors of the method.

Finally, since the determinations of percentage-frequency of cells in different stages of division yield data similar to those given by mitotic counts, the significance of these is considered in the last section.

Technique of seedling culture

As indicated above, we have used intact roots growing from germinating seeds of peas. The seeds are germinated on damp sand at 25° C. for 3 days in the dark. Seedlings with roots about 2 cm. in length are transferred to a perforated metal disk which covers a pneumatic trough which is filled with water to about 2 cm. below the disk. The roots are threaded through the perforations and their tips brought into contact with the fluid in the trough. All the operations involved in setting up the cultures are done in a red light.

The water in the trough is aerated with a stream of air from an air-pump, and the cultures are kept rigidly in the dark under boxes from which all light is excluded. It has been found that the exclusion of light is an important condition for the elimination of 'mitotic periodicity', and the aeration of the medium is necessary for the vigorous growth of the roots. Each culture carries about 120 seedlings.

Observations have been made at 15°, 20°, 25°, and 30° C. The two intermediate temperatures have been obtained in constant-temperature rooms with a variation of ±1·0° C., the lowest in a cellar in which the temperature fluctuates between 14° and 17° C., and the highest in an incubator with a variation of ±0·1° C.

Determination of over-all rate of division

The technique used is that of Brown and Rickless (1949) as modified by Brown and Broadbent (1950). Tips of a constant length are excised from a group of roots, and at the same time a zone of the same length is marked on a second group of roots, the marking being done in red light. The second group is cultured for 24 hours, after which the marked zone which has increased in length is cut off. The two sets of tips are on each occasion transferred to a known volume of 5 per cent. chromic acid. After treatment for 24 hours the tissues are macerated in the acid, and the total number of cells and the number of non-vacuolated cells is determined by the haemacytometer technique. From the differences between the totals the increase in cell number in an average root in 24 hours may be determined. From the determinations of non-vacuolated cells in each suspension the number of these in an average root before and after the period of culture may be calculated, and from these two values the average number of non-vacuolated cells in the root during the period of culture is determined. It is assumed that all the non-vacuolated cells are meristematic cells, and the increment in the total number of cells is divided by the average number of non-vacuolated cells to give a figure which is a measure of the rate of division and represents the number of divisions per meristematic cell per 24 hours. When the reciprocal of this figure is multiplied by 24 the result gives the duration of the over-all cycle in hours. Thus, if the rate is 1·5, the duration of a single cycle is $\frac{1}{1 \cdot 5} \times 24$, or 16 hours.

The marking of the root is done with indian ink which has been evaporated to a thick consistency, and this is applied to the surface of the root with a fine glass needle. The mark made in this way is not washed off when the roots are returned to water.

Errors in the estimate of over-all rate may occur if divisions proceed in other than non-vacuolated cells. In this connexion two possibilities must be considered: (1) that longitudinal divisions continue after vacuolation has commenced, and (2) that secondary thickening starts immediately extension ceases. With regard to the first, stained squash preparations from the growing region have been examined and mitotic figures found only in non-vacuolated cells. With regard to the second, it has been found that within a mature zone which is 1·0 cm. in length there may be an increase of 30,000 cells in 24 hours. This number is large relative to the number formed in the meristem during the same interval, which may be of the order of 150,000. Thus it is necessary to estimate the increment in the total number of cells from as short an initial zone as possible. We have used an initial root length of 5·0 mm., since marks on the root at shorter distances from the tip tend to damage the meristem. The 5·0 mm. length usually increases to about 2·5 cm. in 24 hours, and we have examined the tissue anatomically within this zone and found no evidence of secondary thickening.

Frequency determination

Most previous investigators have determined the relative frequencies of the stages of division by counts on longitudinal sections cut with the microtome. This method, however, is unsatisfactory for the following reasons. It necessarily involves elaborate embedding procedures and is therefore laborious and prolonged. Mitotic figures are not distributed uniformly over the whole area of the meristem, and the zones of different mitotic density differ in extent. It is therefore extremely difficult to obtain for the whole meristem an average figure for the relative frequency in it of any one type of cell. Thirdly, the variation between roots is large, and an average value for several roots is required. This clearly cannot be obtained quickly with the sectioning procedure.

These difficulties are avoided by a modification of the aceto-carmine squash technique. The tips of a number of roots are removed, immersed in acetic alcohol fixative for 10 minutes, and then treated with normal hydrochloric acid at 60° C. for 10 minutes. After washing in water the tips are immersed in aceto-carmine for at least 2 hours. When staining is complete the tips are transferred to a 10-ml. tube containing 2 ml. aceto-carmine of half the concentration of the staining solution. Three glass beads are dropped into the tube, and the open end is closed with a rubber stopper. The tubes are then secured at the periphery of a pulley 14 cm. in diameter, rotating about a horizontal axis. This is rotated slowly for about 2 hours, during which time the agitation of the fluid by the beads tends to separate the tissues of the meristem into separate cells or into small groups of cells which are stained. A drop of the suspension is transferred to a slide and then covered with a large coverslip (50 × 22 mm.). The preparation is mounted on the mechanical stage of a microscope and examined with a $\frac{1}{6}$-in. objective and a ×10 eyepiece. The observations are made along a series of random transects taken across the slide. One edge of the coverslip is brought into the field of the objective and the slide is then moved along the same lateral line until the second edge is brought into view. Along each transect every non-vacuolated cell is entered as an interphase, prophase, metaphase, anaphase, or telophase unit. The process is repeated with different transects until a total of 1,500 to 2,000 cells has been counted. It has been found that a total count of about 1,700 cells gives comparable results with different drops from the same tube. From these figures the percentage number of cells of each type in the sample drop, and therefore in the group of tips, is immediately obtained.

Unlike the chromic acid treatment the aceto-carmine maceration involves a considerable destruction of cells. It is clear, however, that since a percentage figure only is required, the destruction of cells will not affect the result if all cell types are destroyed relatively to the same extent. If the treatment involves a relatively greater destruction of one cell type than of another, this would invalidate the technique. This possibility has been examined by determining the percentage number of cells of each type in a suspension shaken for different periods of time. The results of one experiment are shown in Table I.

TABLE I

Percentages of cells in different stages of division after different periods of mechanical shaking of the same suspension

Division stage	1 hr.	2 hrs.	3 hrs.	4 hrs.
Interphase . . .	88·4	88·8	88·2	90·4
Prophase . . .	8·0	7·6	7·4	6·1
Metaphase . . .	2·1	2·0	2·1	1·2
Anaphase . . .	0·4	0·3	0·8	0·7
Telophase . . .	1·1	1·4	1·5	1·6

It is evident that the destruction at least during the first 3 hours is distributed uniformly throughout all stages.

The general technique would be considerably simplified if the percentage frequency of division stages could be determined with the same cell suspension as that with which the total cell number is estimated. Unfortunately no treatment has yet been found with which complete maceration of mature as well as meristematic tissue can be effected and with which at the same time the staining of the nuclear material can be induced.

Throughout this investigation the criteria for the several stages have been: for prophase, cells showing distinct chromosome threads within a nuclear membrane and which are not associated with other cells in such a way as to suggest late telophase; for metaphase, cells without a nuclear membrane and with distinct chromosomes either randomly distributed or on a metaphase plate; for anaphase, cells without a nuclear membrane but with two groups of chromatids with free chromatid endings showing; for telophase, cells having two distinct nuclei without loose chromatid endings and without a cell-wall membrane between them. Closely associated nuclei which are in a post-telophase stage as here defined have been assigned to interphase.

It may be noted that the microscopical observations involved in making the frequency determinations occupy 20–30 minutes.

Experimental design

Each determination in the series of observations involves a sample of 10 seedlings. For the group of observations for a 24-hour period 30 seedlings are involved. At the beginning of the period a random sample of 20 seedlings from the culture of 120 seedlings is taken: from 10 roots, tips 5·0 mm. in length are removed and transferred to 5 per cent. chromic acid; on the other 10, marks are placed on each root at 5·0 mm. from the tip. This second group is returned to the culture. At the end of the 24-hour period the seedlings with marks on their roots are withdrawn, together with a random sample of 10 seedlings which have not previously been disturbed. The zone between the mark and the tip on each of the 10 roots is removed and transferred to 5 per cent. chromic acid. From the tips removed at the beginning and end of the period and transferred to chromic acid the over-all rate is calculated. From the random sample taken at the end of the period 2·0 mm. tips are removed and transferred to acetic alcohol fixative, and from this material the percentage number of cells in different stages of division is obtained.

The observations are repeated on four successive days with each culture, on each day the operations required at the end of one period and at the beginning of the next being repeated.

A typical set of primary and calculated values assembled for one 24-hour period are as follows:

(*a*)	Total No. of cells at beginning of period	299×10^3
(*b*)	,, ,, end ,,	416×10^3
(*c*)	Meristematic cells at beginning ,,	107×10^3
(*d*)	,, ,, end ,,	109×10^3
(*e*)	Increment in number of cells $(b-a)$	117×10^3
(*f*)	Average No. of meristematic cells $(c+d)/2$	108×10^3
(*g*)	Rate of division (e/f)	1·08
(*h*)	Duration of single cycle $(24/g)$	22·2 hours
	Numbers in transects of (*i*) Interphase	1,660
	(*j*) Prophase	130
	(*k*) Metaphase	28
	(*l*) Anaphase	5
	(*m*) Telophase	25
	(*n*) Total	1,848
	Percentage of (*o*) Interphase $(100\ i/n)$	89·8
	(*p*) Prophase $(100\ j/n)$	7·0
	(*q*) Metaphase $(100\ k/n)$	1·5
	(*r*) Anaphase $(100\ l/n)$	0·3
	(*s*) Telophase $(100\ m/n)$	1·4
	Durations of Interphase $(oh/100)$	19·9 hours
	Prophase $(ph/100)$	1·6 ,,
	Metaphase $(qh/100)$	0·3 ,,
	Anaphase $(rh/100)$	0·07 ,,
	Telophase $(sh/100)$	0·3 ,,

Errors of the technique. It is evident from the data given in the next part of this paper that the differences between values obtained on successive days with the same experimental treatment may exceed 100 per cent. The differences are probably due partly to changing conditions in the roots, since it is probable that the activity of the meristem is affected by the induction of secondary thickening and by the development of laterals. The differences, however, are undoubtedly due primarily to random errors in the technique. The final values incorporate the errors of three sets of independent determinations—the measurement of the increment in the total number of cells, the determination of the average number of non-vacuolated cells, and the determination of the relative frequency of cells in each division stage. The errors involved in the first two have been discussed elsewhere (Brown and Rickless, 1949); the errors in the third may be considered here. It is clear that the error involved in the estimation of relative frequency will vary with the number of each type of cell in the sample. The largest number of any type is always interphase, and the lowest anaphase. The error is correspondingly lowest with interphase and highest with anaphase. Indeed, with anaphase the errors are so large as to make the values obtained of doubtful significance.

Although the errors between individual determinations in the same experimental series are large, the mean of the four determinations in the series has considerable significance. The data of Table II show the results of two duplicate sets of determinations, and it is evident that the errors for interphase, prophase, and metaphase do not exceed 30 per cent. The errors for anaphase and telophase are greater since cells in these stages are usually the least frequent.

TABLE II

Durations (hours) of different stages of division determined in two sets of duplicate experiments. Each value is the mean of four determinations. All cultures given mineral nutrients, A at 25° C. and B at 30° C.

	A		*B*	
Division stage	(1)	(2)	(1)	(2)
Interphase . . .	12·4	14·1	12·7	10·6
Prophase . . .	1·06	0·84	0·77	0·61
Metaphase . . .	0·23	0·20	0·14	0·18
Anaphase . . .	0·04	0·03	0·04	0·04
Telophase . . .	0·23	0·16	0·10	0·12

Limitations of the method

As indicated above, the method rests on the assumption that divisions in the meristem are random. It is clear, however, that they cannot be completely so. On the other hand, the extent of the deviation from strict randomness is not sufficiently great to invalidate the method.

The following possible deviations from strictly random behaviour must be considered: (1) the incidence of mitotic periodicity, (2) the influence of one cell on a neighbouring cell, (3) the relation of daughter cells to each other, (4) the effect of different zones in the meristem.

If division is random, then the frequency of different division stages throughout 24 hours should not vary. On the other hand, various workers (Laughlin, 1919; Kellicott, 1904) have reported that there are diurnal cycles in the frequency of mitotic figures in the meristem, and if these occurred the estimate of the duration of each phase with the technique used here would differ with the time of the day at which the observations are made. Mitotic waves have been observed by us in pea roots when they are exposed to fluctuating diurnal conditions. They do not, however, occur, at least with peas, when the roots are maintained in strictly constant conditions. The data of

Table III show the results of one experiment in which the percentage of cells in different stages of division in roots cultured at 20° C. and kept in darkness, and in 12 hours' light followed by 12 hours' darkness, was determined at intervals of 4 hours. In the second group the light period was from 10 a.m. to 10 p.m. It is evident that while there is little or no change throughout the 24 hours when the roots are kept in darkness, with alternating light and dark a maximum percentage of mitotic figures appears after the beginning of the dark phase.

TABLE III

Percentages of cells in different stages of division at intervals of 4 hours in two series, both cultured at 20° C. Series A in continuous darkness, series B in light from 10 a.m. to 10 p.m. and in darkness for the following 12 hours

	A						*B*					
Division stage	12.0 noon	4.0 p.m.	8.0 p.m.	12.0 mid-night	4.0 a.m.	8.0 a.m.	12.0 noon	4.0 p.m.	8.0 p.m.	12.0 mid-night	4.0 a.m.	8.0 a.m.
Interphase .	90·1	91·3	91·3	91·7	90·2	91·5	90·3	90·5	90·4	86·5	89·6	91·3
Prophase .	6·4	5·7	6·4	6·2	7·2	6·8	5·7	6·8	6·8	9·8	8·0	6·0
Metaphase .	1·8	1·5	1·3	1·2	1·0	0·9	1·9	1·7	1·2	1·6	1·2	1·5
Anaphase .	0·4	0·4	0·3	0·1	0·5	0·2	0·4	0·4	0·3	0·6	0·3	0·3
Telophase .	1·4	1·1	0·7	0·8	1·1	0·5	1·7	1·0	1·2	1·4	0·9	0·9

Wagner (1930) has shown that mitotic figures in the meristem tend to occur in groups, and we have observed the same phenomenon. The position suggests that mitotic activity in one cell may influence a neighbouring cell. This condition, however, would only influence the validity of the technique if the number of cells involved was relatively large. In that case it would tend to promote peak phases of mitotic activity and these phases, as the data of Table III show, do not occur in constant conditions.

One daughter cell of a division is more likely to divide at the same time as the other daughter than with a non-related cell. Thus, if the products of a division accumulated in the meristem, families of cells would be produced which would tend to divide together. Since, however, the number of cells formed in 24 hours may be of the order of 150,000, and since the number of meristematic cells is not usually greater than 120,000, it is evident that at least half the cells formed during a 24-hour period must vacuolate, and the accumulation of families of cells must therefore be comparatively restricted.

One important non-random condition may be involved which would not be shown by data such as those of Table III and which might influence the estimates of duration. The strict random condition would require that the probability of a cell entering the mitotic phase during any time interval is the same in all parts of the meristem. Such it clearly is not. Wagner (1930) has shown that the frequency of mitotic figures tends to be greater in the zone immediately behind the root-cap than it is at the limit of the meristem. This characteristic of the meristem may be disregarded if changes in the number of cells in the meristem affects all zones to the same relative extent. If it does not, and if, for instance, a reduction in the total number of meristematic cells were to occur only at the expense of the zone farthest from the tip in which the frequency of mitotic figures is lowest, then the method might tend to give exaggerated estimates of duration when the meristematic number of cells is low. This possibility has been examined by determining the frequency of various division stages when the number of meristematic cells decreases with constant experimental conditions. This decrease occurs particularly rapidly with high temperatures, and in these conditions, if the decrease occurs only at the expense of the zone with low mitotic frequencies, then the percentage of various mitotic phases should increase with time. The data of Table IV show that they do not, and the decrease in cell number therefore probably affects all regions of the meristem to the same relative extent.

TABLE IV

Number of meristematic cells and percentages of cells in different stages of division. Observations made on four successive days with culture maintained at 30° C. in the dark

	1	2	3	4
Number of meristematic cells	102,000	83,000	43,000	32,000
Division stages:				
Interphase . . .	92·7	91·6	94·0	92·3
Prophase . . .	4·5	5·1	4·2	5·2
Metaphase . . .	1·8	1·6	0·9	1·0
Anaphase . . .	0·4	0·2	0·2	0·1
Telophase . . .	0·7	1·6	0·8	1·4

Although the data of Table IV indicate that the occurrence of a gradient of mitotic frequency is not affected by changes in the number of meristematic cells, the fact that the gradient is a characteristic of the meristem carries an important implication. The durations are calculated from average figures for the whole meristem and they do not therefore represent the precise times for any particular type of cell from a particular point of the meristem.

Finally, two important limitations of the technique may be noticed. Since observations on percentage frequency are only made on one occasion during the 24 hours, the method is only applicable when it can be assumed that the position at the time of observation is representative of the whole 24 hours, and the method can therefore be used only when conditions are constant and when rapid changes are not occurring in the meristem.

It has been shown above that duplicate determinations of the percentages of different types of cells in a suspension agree closely. The agreement, however, may not be as close as that shown in Table I, when different observers are involved. The differences in the relative values obtained by different observers is not, however, large, and they do not, of course, affect the comparative significance of the data when all the observations have been made by the same observer.

Significance of mitotic counts

A number of workers (v. Guttenberg, 1928; Gurwitsch, 1929; Hammett, 1929) have assumed that counts of the number of mitotic figures in sections through the meristem provide a measure of the relative rate of division. It has been assumed that the larger the number given by the count the greater the rate of division. It is clear, however, that the results of such counts, even when they are expressed in terms of the percentage number of cells in mitosis, could only be a measure of the relative rate if the duration of mitosis is constant whatever the treatment. The results of this investigation, however, show that the duration of mitosis may vary as much as that of interphase.

Clearly the number of mitotic figures in a system such as that of the root meristem is determined by the relative durations of mitosis and of the over-all cycle, and is given by a ratio which may be expressed as $M/(I+M)$, where M is the duration of mitosis and I that of interphase. The number of mitotic figures present at any one time will increase as the value of this ratio increases. But the value may increase either by decreasing the length of interphase or by increasing the length of mitosis. In the first case the over-all cycle is shortened, in the second it is lengthened. Thus an increase in the number of mitotic figures may occur either with an increase or with a decrease in the length of the over-all cycle. Similarly the occurrence of the same number of mitotic figures does not indicate the same rate of division. If a particular treatment has the same relative accelerating or retarding effect on interphase and mitosis, then the number of mitotic figures will not change.

Results

In this section the results obtained with each phase of division are presented separately. A statistical analysis of the data is provided with each table, and since the analyses show that the inorganic nutrients have given a significant effect only with telophase, over-all means for the eight observations in each temperature treatment have been calculated and these are shown at the base of each table. Finally, the over-all means are collected in the last table of the series.

TABLE V

Effect of temperature and inorganic nutrients on duration (hours) of interphase. N− indicates water and N+ a solution of mineral nutrients

	15° C.		20° C.		25° C.		30° C.	
	N−	N+	N−	N+	N−	N+	N−	N+
	29·1	21·4	23·3	20·0	10·3	14·9	9·2	8·8
	19·3	28·8	13·7	10·7	19·2	8·7	12·4	11·4
	18·7	29·3	16·5	15·4	20·7	14·4	26·5	13·6
	17·9	16·0	15·5	21·9	16·0	11·4	15·6	8·7
Means	22·6		17·0		14·5		13·3	

Variance due to:	d.f.	M.S.	V.R.	P.
Temperature .	3	136·64	5·4	<0·01
Nutrients .	1	25·38	1·0	—
Interaction .	3	26·65	1·05	—
Error . .	24	25·32	—	—

Interphase. It is evident from the statistical data that accompany Table V that temperature gives a highly significant effect with interphase but that nutrient treatment does not. There is an accelerating effect as temperature is raised from 15° to 25° C. Further increase in temperature has little effect. The ratio of the durations at 15° and 25° C. is 1·6.

Prophase. It is evident from the statistical data that accompany Table VI that the effect of temperature on prophase is highly significant but that the effect of nutrients is not. There is an accelerating effect as temperature is raised from 15° to 25° C., the ratio of the durations at the two temperatures being 2·3.

TABLE VI

Effects of temperature and nutrients on duration (hours) of prophase. Symbols as in Table V

	15° C.		20° C.		25° C.		30° C.	
	N−	N+	N−	N+	N−	N+	N−	N+
	1·69	1·77	1·65	1·56	0·57	1·04	0·45	0·60
	2·16	2·46	0·61	0·65	0·89	1·00	0·69	0·54
	1·52	3·47	0·75	1·01	0·90	1·31	1·17	0·63
	1·47	1·95	1·60	1·92	0·92	0·90	0·88	0·67
Means	2·1		1·3		0·9		0·70	

Variance due to:	d.f.	M.S.	V.R.	P.
Temperature .	3	2·804	15·41	<0·01
Nutrients .	1	0·396	2·18	>0·05
Interaction .	3	0·271	1·49	>0·05
Error . .	24	0·182	—	—

Metaphase. The statistical data that accompany Table VII show that the effect of temperature on metaphase is highly significant but that the effect of nutrients is not. There is an accelerating effect with increase of temperature, the ratio of the durations at 15° and 25° C. being 1·7.

TABLE VII

Effects of temperature and nutrients on duration (hours) of metaphase. Symbols as in Table V

	15° C.		20° C.		25° C.		30° C.	
	N−	N+	N−	N+	N−	N+	N−	N+
	0·44	0·43	0·39	0·34	0·26	0·32	0·18	0·20
	0·33	0·59	0·15	0·23	0·28	0·13	0·21	0·15
	0·25	0·45	0·22	0·21	0·24	0·25	0·25	0·20
	0·40	0·25	0·23	0·13	0·19	0·21	0·17	0·16
Means	0·40		0·24		0·24		0·19	

Variance due to:	d.f.	M.S.	V.R.	P.
Temperature .	3	0·0627	9·95	<0·01
Nutrients .	1	0·0001	—	—
Interaction .	3	0·0079	—	—
Error . .	24	0·0063	—	—

Anaphase. The statistical data that accompany Table VIII show that neither the temperature nor the nutrient effects are significant. Above, it is shown that an abnormally high error might be expected in the estimation of the anaphase duration, but nevertheless the data suggest that there may be a temperature effect, and this conclusion is accepted tentatively.

TABLE VIII

Effects of temperature and of nutrients on duration (hours) of anaphase. Symbols as in Table V

	15° C.		20° C.		25° C.		30° C.	
	N−	N+	N−	N+	N−	N+	N−	N+
	0·15	0·09	0·12	0·06	0·03	0·03	0·04	0·02
	0·08	0·07	0·03	0·05	0·07	0·02	0·03	0·04
	0·02	0·09	0·06	0·06	0·02	0·08	0·05	0·01
	0·08	0·05	0·04	0·13	0·09	0·04	0·01	0·07
Means	0·08		0·07		0·05		0·04	

Variance due to:	d.f.	M.S.	V.R.	P.
Temperature .	3	0·0033	3·0	>0·05
Nutrients .	1	0·0001	—	—
Interaction .	3	0·0005	—	—
Error . .	24	0·0011	—	—

Telophase. The statistical data that accompany Table IX show that both the nutrient and the temperature effects in telophase are significant, the first at the 5·0 per cent. and the second at the 1·0 per cent. level. Temperature has an accelerating effect, the ratio for the durations at 15° and 25° C. being 1·9. The inorganic solutes tend to depress telophase. The effect, however, is not large, and over-all means for the temperature treatments have therefore again been calculated.

TABLE IX

Effects of temperature and nutrients on duration (hours) of telophase. Symbols as in Table V

	15° C.		20° C.		25° C.		30° C.	
	N−	N+	N−	N+	N−	N+	N−	N+
	0·48	0·36	0·30	0·30	0·18	0·28	0·07	0·15
	0·16	0·53	0·05	0·12	0·24	0·09	0·21	0·10
	0·27	0·50	0·09	0·35	0·11	0·33	0·23	0·10
	0·28	0·35	0·18	0·31	0·11	0·20	0·24	0·11
Means	0·37		0·22		0·19		0·16	

Variance due to:	d.f.	M.S.	V.R.	P.
Temperature .	3	0·0706	7·93	<0·01
Nutrients .	1	0·0300	3·37	<0·05
Interaction .	3	0·0177	1·99	>0·05
Error . .	24	0·0089	—	—

Summary of results. The over-all means for each temperature with each stage of division are assembled in Table X.

TABLE X

Mean durations (hours) for each temperature for each stage of division

Division stage	15° C.	20° C.	25° C.	30° C.
Interphase . . .	22·6	17·0	14·5	13·3
Prophase . . .	2·1	1·3	0·9	0·7
Metaphase . . .	0·40	0·24	0·24	0·19
Anaphase . . .	0·08	0·07	0·05	0·04
Telophase . . .	0·37	0·22	0·19	0·16

DISCUSSION

The values for the durations of the several phases given in the last section may be compared with those given by earlier workers. Laughlin (1919) measured the durations of the different phases in the root-tip of the onion. The limits for the various phases used by this worker are in some respects different from those used by us, but from the data given by Laughlin the durations of the different phases as defined by us may be calculated, and they are for 20° C.; interphase, 160 minutes; prophase, 70 minutes; metaphase, 4 minutes; anaphase, 1·5 minutes; telophase, 1·5 minutes. The corresponding values obtained by us with pea roots are: interphase, 17 hours; prophase, 80 minutes; metaphase, 15 minutes; anaphase, 4 minutes; telophase, 13 minutes. The differences between the two sets of results are considerable and they may indicate that the position differs in the roots of different species. It is proposed to examine the process in the onion with the technique of this investigation.

Huskins and Steinitz (1948) have reported that the duration of the division cycle in the root is 24 hours. This value may be compared with those recorded here for single cycles at 15° C. and 20° C. which are respectively 26 and 19 hours. Barber (1939) has measured the duration of the various phases of mitosis in hairs of *Tradescantia* by observing the process microscopically, and gives the following values for 20° C.: prophase, 3–4 hours; metaphase, 15–25 minutes; anaphase, 25 minutes; and from the end of anaphase to the formation of the wall membrane, 4–7 minutes. The corresponding values obtained by us at 20° C. are: prophase, 1 hour 18 minutes; metaphase, 14 minutes; anaphase, 5 minutes; anaphase to wall formation, 13 minutes. Clearly, except for the anaphase figures the agreement between the two sets of data is reasonably close, especially in view of the different materials involved in the two sets of observations, and of the different conditions of observation.

It is evident from the data that all stages of division are accelerated with increasing temperature in the intact root of the pea over the range of temperatures used here. The acceleration is greatest over the range from 15° to 25° C. The ratios of the durations at the two temperatures suggest Q_{10} values of 2 or slightly less. The position suggests that at all stages between these two temperatures the rate is being controlled by enzyme-dependent processes. This is probably the case with prophase and telophase, in which the Q_{10} values are 2·4 and 1·9 respectively, and possibly the case with interphase and metaphase, for which the recorded values are 1·6 and 1·7 respectively. Between 25° and 30° C. there is little change in duration with increase in temperature, and over this range conditions other than metabolic changes are evidently limiting the rate.

Clearly with the material used in this investigation, since each stage is accelerated with increase in temperature from 15° to 30° C., the over-all rate is also correspondingly increased. On the other hand, Brown and Rickless (1949) found that with increase in temperature from 15° to 25° C. the over-all rate decreased. These workers, however, were using small apical fragments of *Cucurbita*, which did not carry mature tissue, and the nutrient for which was supplied from an external medium. The nature of the difference in the experimental situation which promoted the different response has not been determined. It may be noted in this connexion, however, that attachment of the meristem to mature tissue is an important condition for division. It has been shown (Brown and Whiteman, 1950) that at least some of the metabolites required for division are synthesized in mature tissue and transported to the meristem. In the present connexion this conclusion carries a further important implication. It suggests that some of the enzyme-dependent processes involved in division may not be localized in the cells of the meristem but may proceed in other parts of the root.

The absolute values for the several stages of division are of some importance in relation to the significance for growth of effects in the different stages of division. Since mitosis occupies a relatively small proportion of the whole cycle, depressing or accelerating effects exerted during mitosis will not affect growth to any considerable extent. On the other hand, interphase normally occupies about 90 per cent. of the whole cycle, and treatments which accelerate or depress this phase will necessarily have a very pronounced effect on growth. In connexion with the present series of studies it is evident that when temperature accelerates growth and when this is due at least partly to an effect on division, this effect must be referred primarily to the influence of temperature on interphase.

We have to acknowledge the considerable help and advice given in this investigation by Professor I. Manton; also the help of Miss Townsend, who made many of the observations that have been recorded.

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Response of Stomata to Subnormal Carbon Dioxide Concentrations

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In a preliminary note in *Nature* (vol. **161**, p. 179, 1948) and in two recent papers in this Journal (vol. **1**, pp. 29 and 227, 1950) I described work leading to the finding that stomata of *Pelargonium* and of wheat respond markedly to small changes in the carbon dioxide content of the air below the normal 0·03 per cent.; I have now for the first time seen a paper by Freudenberger (*Protoplasma*, **25**, 15, 1940) in which a similar discovery (in qualitative terms) is announced for *Canna* and some other genera. I much regret that owing to the war the volume of *Protoplasma* in question has not been available and I was thus unable to refer to this interesting paper. This work of Freudenberger's will be discussed in relation to my own in due course.

November, 1950.

The Wheatstone Bridge Porometer

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THE main trend of porometer design has been to improve ease and rapidity of reading: thus the original porometer of Darwin and Pertz (1911), the Knight porometer (1915), and the 'resistance porometer' of Gregory and Pearse (1934), if calibrated in absolute terms, are all capable of yielding the same information, viz. leaf conductance or resistance to viscous air flow, but they represent successive improvements in convenience. Sensitivity is so much a matter of dimensions that comparisons between the different designs are difficult to make, but it seems probable that the Knight porometer is the least sensitive. A notable exception quite outside this main trend is provided by the 'diffusion porometer' of Gregory and Armstrong (1936) which yields information of much more fundamental importance but is certainly less convenient. The slight modification made in the resistance porometer by Heath (1941) and the more extensive alterations made by Spanner and Heath (1951) had for their object the reduction of various errors, although in the latter case the use of a 'gasometer' type of aspirator and manometers filled with paraffin also resulted in greater ease and accuracy of reading. The new porometer described below represents, it is thought, a further advance in the same direction, having proved in use to be of greater convenience than any other; it can also be made to give reasonably rapid and sensitive responses over an extremely wide range of leaf resistance. It has been called the 'Wheatstone bridge porometer' since it is in fact nothing but a conventional Wheatstone bridge, with pneumatic pressures in a system of capillaries substituted for electrical potentials in electrical conductors. The Wheatstone bridge principle applied to air flow has been employed previously in this Institute (Porter, Pal, and Martin, 1950) for standardizing capillary resistances. In the present apparatus a null method of reading is made possible by the use of a continuously variable resistance—in this case a fine control needle valve. The apparatus is shown diagrammatically in Fig. 1.

The aspirator. A 'gasometer' type aspirator working in medicinal paraffin (Spanner and Heath, 1951) can be used to provide either a negative or positive pressure difference, but the latter method is preferable for reasons discussed in the paper cited. The aspirator may then be used to supply moist, but not saturated, air to the system, thus reducing errors due to augmentation of the mass flow with water vapour during passage through the leaf. The 'circuit' is completed by the atmosphere. Since air escapes through both pairs of arms of the bridge, at low resistances the aspirator requires rather frequent replenishment. It is therefore convenient to effect this through the tube *t*, by means

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of a small pressure, without the necessity for interrupting the use of the porometer. A constant pressure aspirator is not essential as long as any pressure changes are slow, and for field work a rubber bladder suitably weighted can be substituted for the aspirator.

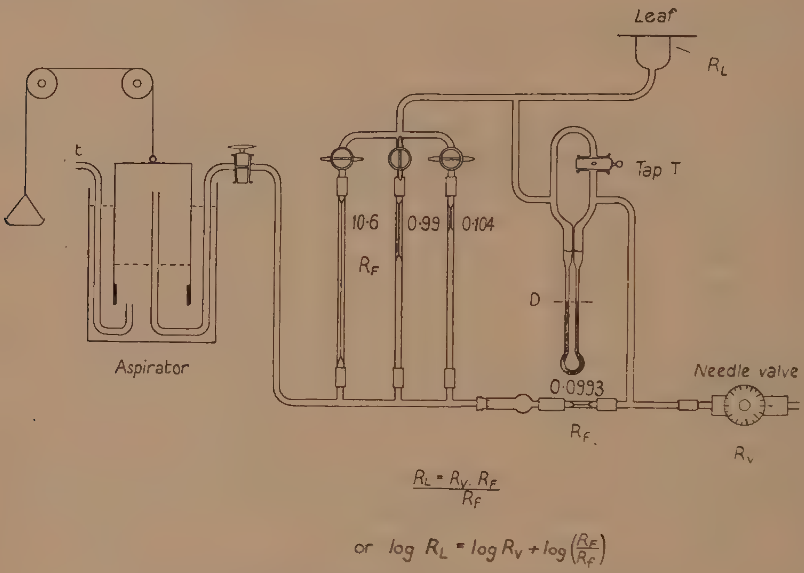
Leaf
R_L
t
Tap T
10·6
0·99
0·104
R_F
D
Aspirator
Needle valve
0·0993
R_f
R_v
$R_L = \frac{R_v . R_F}{R_f}$
or $\log R_L = \log R_v + \log\left(\frac{R_F}{R_f}\right)$

FIG. 1. Diagram of apparatus. For explanation see text.

The variable resistance, R_v. The needle valve (Edwards & Co., Type L.B.1) was calibrated by means of a resistance porometer in terms of 'Gregory and Pearse units'.[1] The actual experimental determinations, in terms of $\log_{10} R_v$, are plotted against position of the needle-valve dial in Fig. 2 together with a freehand curve. The good agreement between the duplicate determinations, which were made with the dial turning in opposite directions, shows the absence of back-lash and the reproducible results obtainable with this needle valve. The sensitivity may be considered satisfactory between 2 and 10 revolutions of the dial, i.e. over a 100-fold range of resistance from 0·008 to 0·88 units.

The fixed resistances, R_f *and* R_F. The use of three alternative calibrated capillary resistances at R_F (approximately 0·1, 1·0, and 10·0 units) enables a 10,000-fold range of leaf-resistance R_L to be covered. This total range could be extended to 1×10^6 by using three values of R_F spaced apart 100× instead of 10× (p. 114 below). To give the best over-all sensitivity the value of R_f should be chosen in the middle of the useful range of the needle valve, but the somewhat higher value used (0·1 units) reduces the rate of depletion of the air in the aspirator by restricting the flow through the arms of the bridge R_f R_v It will be noted from Fig. 1 that a drying tube is fitted before R_f. There is no

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[1] Unit resistance equals that of a cylindrical tube for which

$$l/r^4 \doteqdot 3{\cdot}77\times10^8 \text{ cm.}^{-3} \text{ (Heath, 1939).}$$

objection to passing dry air through the arms R_f R_v and moist air through the arms R_F R_L since the ratios R_f/R_v and R_F/R_L will be unaltered by the small difference in viscosity of the air flow through the two pairs of arms.

The manometer. This corresponds to a galvanometer of infinite resistance, in the electrical analogy, since there is no flow. A compound manometer is used, with Brodie's fluid in the narrow part of the tube and light medicinal paraffin (s.g. 0·83) extending up into the wide bore tubing. In such a manometer, if the ratio of the two cross-sectional areas is large, the readings of the menisci where the two liquids meet approximate to those in a manometer filled with a single liquid of density equal to the difference of the densities of the two liquids used. In the present case this is 1·03–0·83 or 0·20; thus a 5-fold increase in sensitivity as compared with a water manometer is obtained. The liquids usually suggested for such manometers, e.g. phenol-in-water and water-in-phenol, all have the disadvantage for work with stomata of possessing high vapour pressures, often of toxic substances. Not only is the medicinal paraffin free from this defect, but the meniscus with the Brodie's fluid is extremely stable and easy to read. The tap T across the manometer corresponds to a 'shunt' and is opened temporarily when the aspirator pressure is first applied to the porometer in order to avoid the initial lack of balance due to faster flow along the pair of arms having the lower resistance. This tap is also opened to enable the movable datum line D to be set to the null position of one meniscus in the manometer. This meniscus and D are observed through a magnifying lens with a peep-hole to reduce parallax errors.

The porometer cup. For reasons discussed elsewhere (Heath, 1950; Spanner and Heath, 1951) a detachable cup, or at least one swept between readings with air such as surrounds the rest of the leaf, should always be used with this as with any other porometer.

The method of use. To take a reading the detachable cup (Spanner and Heath, 1951) is adjusted to make an airtight seal with the portion attached to the leaf, and the aspirator pressure is applied to the porometer with tap T open. T is then closed and the dial of the needle valve rotated until the meniscus returns to its null reading at D. From the calibration curve the value of log R_v corresponding to the reading of the needle valve dial position is obtained. Hence the log (leaf resistance) is obtained from

$$\log R_L = \log R_v + \log(R_F/R_f).$$

For best sensitivity the value of R_F should be selected which lies nearest to R_L.

Sensitivity and speed of response. The relative sensitivity may be defined as the smallest fractional or percentage change in the leaf resistance R_L which can be easily detected. Since $R_L = R_v(R_F/R_f)$, this is the same as the smallest easily detectable relative change in the needle-valve resistance R_v and this depends upon the smallest movement of the manometer meniscus which can be easily observed. The latter may be taken as 0·2 mm. It can be shown theoretically that the fractional change in R_v necessary to throw the bridge out of balance by such a minimal amount is independent of the absolute values of the resistances R_F, R_L, R_f, and R_v but changes with their ratios. It is minimal when R_F/R_L $(= R_f/R_v) = 1$ and is approximately doubled when this ratio has a value of 6. As stated above, therefore, R_F should be chosen near in value to R_L to give best sensitivity. Furthermore, for small manometer readings, the fractional change in R_v needed to give the smallest easily detectable reading is directly proportional to that reading and inversely proportional to the aspirator pressure. Thus the precision with which relative changes in R_L can be detected improves in direct proportion to the sensitivity of the manometer and to the aspirator pressure. This emphasizes the desirability of using a sensitive manometer and the highest convenient pressure in the aspirator.[1]

The sensitivity was determined empirically using each of the three resistances R_F in turn and at three selected values of R_v to cover the whole range of leaf-resistance R_L for which the apparatus could be used. The results are presented in Table I. The method was to use a second needle valve instead of a porometer cup at R_L and to measure with a reading microscope, for each combination of R_F and R_v, the movements of one meniscus in the manometer due to various small movements of the needle-valve dial R_v away from the position giving perfect balance. Hence by interpolation the number of dial divisions movement causing a 0·2 mm. rise of one meniscus was found (*a* in Table I). This number of divisions multiplied by the slope of the calibration curve (Fig. 2) at the appropriate point, expressed as change of $\log R_v$ per division, gave directly the absolute sensitivity in terms of $\log R_L$ and hence the relative sensitivity in terms of R_L. These values are tabulated as percentage sensitivity in Table I (*b*) and it will be seen that they nowhere much exceed 2 per cent. As predicted by theory the sensitivity is (approximately) constant for any given value of R_v, i.e. when R_f/R_v is constant; also, the apparatus is less sensitive when R_f/R_v is very small or very large, as when R_v is 0·88 or 0·0079, and the sensitivity improves as R_f/R_v approaches unity (R_v, 0·029).

The time of response (Table I *c*) was determined as the time to reach 80 per cent. of the full manometer reading when the bridge was put out of balance by various small amounts after perfect balance had been attained. This time was found to be more or less independent of the degree of unbalance. For the aspirator pressure used, therefore, the tabulated times represent the order of the lag in observing the existence of a barely detectable change in leaf resistance. These times would vary inversely with the aspirator pressure, and this provides an additional reason for using a relatively high pressure.

As already stated, the total range of leaf resistance covered by the apparatus as set up is about 10,000×, and this is indicated by the values *d* of R_L tabulated. It will be seen that this range could be covered, by eliminating almost all overlap, with two values of R_F only, viz. 0·104 and 10·6. Thus as stated earlier, three values of R_F spaced in steps of 100× instead of 10× should enable a million-fold range to be covered and the sensitivity should nowhere be much worse than 2 per cent. Since this sensitivity is adequate for most purposes it may be doubted whether the more closely spaced values of R_F are worth while, although they do enable sensitivity to be improved in the middle of the range by choosing an R_F close to R_L and a 10,000-fold range has been found more than sufficient in practice.

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[1] The authors are indebted to Mr. D. C. Spanner for the above theoretical considerations.

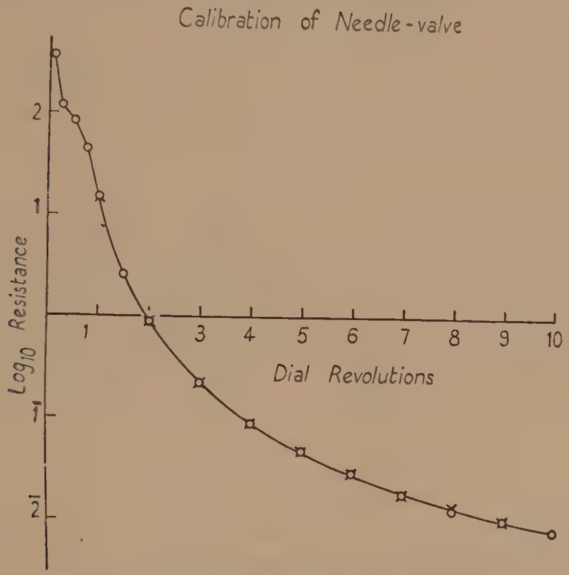

FIG. 2. Calibration curve for the needle valve.

TABLE I

Sensitivity and response time with 7·5 cm. of paraffin pressure in aspirator

R_f = 0·099 units

		R_F		
R_v		0·104	0·99	10·6 units
0·88 units	*a*	0·9	1·1	1·2
(2 revs.)	*b*	1·4%	1·7%	1·9%
	c	20 sec.	16 sec.	65 sec.
	d	0·92	8·7	93·9
0·029 units	*a*	1·7	1·8	1·8
(6 revs.)	*b*	0·8%	0·9%	0·9%
	c	7 sec.	7 sec.	16 sec.
	d	0·030	0·29	3·1
0·0079 units	*a*	10	13	10
(10 revs.)	*b*	1·8%	2·3%	1·8%
	c	2 sec.	1 sec.	1 sec.
	d	0·0083	0·078	0·84

a = number of dial divisions (hundredths of a revolution) for 0·2 mm. rise of one meniscus above datum line.
b = Percentage sensitivity (in terms of R_L).
c = Time taken to reach 80% of final response.
d = Leaf resistance R_L in Gregory and Pearse units.

Advantages

1. The calculation of leaf resistance does not involve the aspirator pressure, which thus need not be constant as long as rapid changes do not occur during a reading. A weighted rubber bladder can therefore be used for field work, as already mentioned.

2. Only one manometer need be observed and that merely as a null-point detector. The range of movement of the meniscus required is thus small; hence the response is rapid, a manometer of great sensitivity may be used, and a fixed magnifying lens is adequate. (A second manometer is, however, useful as a check on the aspirator pressure.)

3. The arms R_F, R_L of the bridge, together with the plant, may be arranged in a constant temperature chamber at one temperature while the arms R_f, R_v and the manometer are outside the chamber at another temperature. In this case sufficient tubing in the chamber should precede R_f for the air to have reached the controlled temperature before passing through the resistance.

4. The apparatus can be made self-recording by standard methods for null-point instruments. The use of recording porometers, however, involves complications due to the need for detaching or sweeping the cup between readings.

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Studies on Nitrogen Fixation by Blue-green Algae

II. NITROGEN FIXATION BY *MASTIGOCLADUS LAMINOSUS* COHN

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SUMMARY

Mastigocladus laminosus Cohn, a blue-green alga of hot springs, has been obtained in pure bacteria-free culture and has been shown to be capable of fixing elementary nitrogen. This appears to be the first instance in which this capacity has been demonstrated in a blue-green alga belonging to a family other than the Nostocaceae.

ALTHOUGH a capacity to fix elementary nitrogen has been demonstrated in a number of blue-green algae, much remains to be learnt of the distribution of this property within the group. The information hitherto available has suggested that among the Myxophyceae nitrogen fixation may be confined to a single family, the Nostocaceae (Fogg, 1947), evidence of fixation of nitrogen by algae belonging to families other than this being equivocal. Singh (1942) has given satisfactory proof of its occurrence in a species of *Aulosira*, a genus which Geitler (1932) placed in the Microchaetaceae. However, the taxonomic position of *Aulosira* is uncertain, and Fritsch (1945) has considered it to be most appropriately included in the Nostocaceae. The identification of an alga, thought to be *Phormidium molle* (Oscillatoriaceae), which was isolated in pure culture and shown to be nitrogen-fixing by Allison and Morris (1930), is not certain and it appears likely that it was a species of *Anabaena* (Nostocaceae). Copeland (1932) reported that various species of Oscillatoriaceae were able to fix nitrogen, but gave no indication that the cultures that he used were pure. It appears certain that many members of this family do not possess the property; for example, De (1939) was unable to demonstrate fixation in *Phormidium foveolarum* and the several species of *Oscillatoria* and *Lyngbya* examined by me have proved incapable of growth in media free from combined nitrogen. Among the Chroococcales, *Gloeocapsa minor* has been reported as fixing nitrogen (Odintzova, 1941), but the evidence given in support of this assertion is unsatisfactory since pure cultures of the organism were not isolated.

Nevertheless, there is no lack of indications that some blue-green algae other than Nostocaceae possess the property, and it is the purpose of this paper to present definite evidence of nitrogen fixation by an alga belonging to the Stigonemataceae, a family in which the property does not appear to have been recorded previously.

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The alga concerned was abundant in several samples, for which I am grateful to Mr. A. C. S. Wright of the New Zealand Soil Bureau, from the hot springs of the Whakarewarewa native reserve, Rotorua, New Zealand. Professor F. E. Fritsch, who has examined material from cultures isolated from one of these samples, has found the alga to correspond to *Mastigocladus laminosus* Cohn (*Hapalosiphon laminosus* Hansg.). Although this genus has been placed in a separate family by Geitler (1932), both Frémy (1936) and Fritsch (1945) consider it to be best referred to the Stigonemataceae. *M. laminosus* is a characteristic alga of hot springs; accounts of its morphology and biology and references to the literature concerning it have been given by Frémy (1936) and Fritsch (1945).

Placed in a medium free from combined nitrogen *M. laminosus* grew well at room temperatures. Löwenstein (1903) found that this species is capable of considerable adaptation and that it can be cultured at temperatures much lower than those at which it normally grows. The medium used for the culture of *M. laminosus* in this investigation was that used for *Anabaena cylindrica* (Fogg, 1949) with the addition of 0·2 g./litre of sodium bicarbonate. The pH of the medium with this addition is about 8·2. Unialgal cultures which were, however, still contaminated with bacteria, were raised from single filaments isolated by means of a capillary pipette. From one of these, pure bacteria-free cultures were obtained by treatment with ultra-violet light by a method similar to that of Gerloff, Fitzgerald, and Skoog (1950).

The purity of the cultures obtained has been demonstrated both by direct microscopical examination and by inoculation into bacteriological media. The media used were the following: caseinate agar, Lemco-peptone agar, dextrose-peptone broth (for details of the composition of these three media see Fogg, 1942), and the mineral medium used for the culture of *Anabaena* (Fogg, 1949) with the addition of 2 per cent. of mannitol, a solution which has been found particularly suitable for the growth of *Azotobacter*. Inoculation into these was made from an algal suspension prepared by shaking with glass beads (Fogg, 1942). Test cultures were always prepared in triplicate. Bacterial contamination as indicated by the appearance upon incubation of turbidity in liquid cultures or colonies on solid media was absent in all cases.

M. laminosus in pure culture has been found to grow as vigorously in the absence of added combined nitrogen as it does in similar media with the addition of 0·2 g./litre of potassium nitrate. It therefore appears that this alga is able to fix nitrogen. Conclusive evidence of nitrogen fixation has been obtained in an experiment in which an increase in combined nitrogen in medium initially free from combined nitrogen has been demonstrated under conditions excluding the possibility of absorption of nitrogen in a combined form from the atmosphere. The method and apparatus used were essentially those described and discussed in the previous paper of this series (Fogg, 1942) with certain modifications and improvements. The culture vessels used in this investigation were 500-ml. 'Hysil' Drechsel bottles fitted with sintered glass distributors (pore size 40–90μ) and containing 400 ml. of medium. They were arranged in a water-bath maintained at 25·0° C. in a mean light intensity of about 7,000 metre-candles from a 150-watt incandescent-filament lamp. The cultures were aerated with a gas mixture containing 0·5 per cent. of carbon dioxide obtained by mixing air from a diaphragm pump with air containing 5 per cent. of carbon dioxide from a cylinder, the composition and rate of flow of the mixture being controlled with the aid of calibrated flow-meters. Traces of oxides of nitrogen and ammonia were removed by washing successively with a 1 per cent. sodium bicarbonate solution and 25 per cent. (v/v) sulphuric acid. The gas washing bottles used were similar to those used for culture vessels, the rate of flow through them being 40 litres/hour. The culture vessels, of which there were four arranged in parallel, were each fitted with a sterile cotton-wool filter and a wash bottle containing sterile water. Traps containing mercuric chloride solution prevented contamination via the outlets. The culture vessels, together with their filters and traps, were sterilized and assembled under aseptic conditions. Three of them were inoculated with a suspension of *M. laminosus* prepared from a 25-day-old culture in medium free from combined nitrogen. The fourth was left un-inoculated as control. After 20 days there was a quantity of healthy growth in the inoculated vessels, mainly in the form of spherical colonies, *c.* 2 mm. in diameter, on the bottom, and the medium had become a pale lilac colour, which was, however, not due to phycobilins. To ensure that the cultures had not become contaminated during the course of the experiment a small amount of material (less than 0·01 mg. total nitrogen) from each bottle was examined by the methods described above. No contamination by bacteria or fungi was found. The alga and medium from each culture were separated by filtration through sintered glass (pore size 5–15μ) and their total nitrogen contents determined by micro-Kjeldahl analysis using a selenate catalyst mixture in the digestion. The small amounts of alga which had grown into the pores of the sintered glass were recovered by extraction with strong sulphuric acid.

The results of this experiment are set out in the accompanying table and show clearly that free nitrogen has been fixed by the alga.

TABLE I

Nitrogen fixation by Mastigocladus laminosus *Cohn. Nitrogen in mg./400 ml. medium after 20 days' growth. Nitrogen in inoculum = 0·063 mg. per culture*

	Alga	Medium	Total
Control	—	0·047	0·047
1	9·71	3·17	12·88
2	6·27	2·26	8·53
3	6·12	2·37	8·49

The mean net fixation, 2·48 mg./100 ml. of medium, was distinctly less than that which can be accomplished in the same period by *Anabaena cylindrica* growing under similar conditions, i.e. about 17·5 mg./100 ml. of medium.

As in the case of *A. cylindrica* (Fogg, 1942), a large proportion, about 26 per cent., of the nitrogen fixed appears in the medium in a soluble form.

I am grateful to Miss D. Cuthbert for technical assistance in this work.

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The Sugars and Non-Volatile Organic Acids of some Archegoniates: A survey using Paper Chromatography

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SUMMARY

This survey shows that glucose, fructose, and sucrose are the principal sugars of bryophytes and pteridophytes. Free pentoses were not detected and can be present only in very small amounts. The occurrence of raffinose, or an allied sugar, in certain bryophytes, in *Psilotum*, and in some of the more primitive families of ferns is worthy of note. No other sugars were detected in quantity.

Notwithstanding the low pH values there is a striking absence of non-volatile organic acids from the bryophytes. The pteridophytes as a group are also apparently of low acid content, but the Equisetinae have considerable quantities of malic acid. *Isoetes* and *Platycerium* are very rich in malic acid and are indeed comparable in acid content with acid succulents such as *Bryophyllum* and *Sedum*.

There is no evidence of any notable difference between the carbohydrate metabolism of the bryophytes and pteridophytes and that of higher plants, but the acid metabolism, particularly of the bryophytes, may show interesting differences and merits further investigation.

It is usually assumed that the metabolism of the Bryophyta and Pteridophyta is not different in essentials from that of the Spermatophyta, but a search of the literature has revealed slight evidence in support of this view. There is little information even as to the principal organic constituents of these plants.

In an earlier work (Allsopp, 1948) paper chromatography provided a convenient method for investigating the amino-acids of a number of archegoniates. In the present work similar methods have been used in making a survey of the sugars and non-volatile organic acids of a considerable range of these plants.

METHODS AND MATERIALS

Water extracts were prepared by crushing 0·25 g., or less, of the plant material with an equal amount of water. The mixture was raised to boiling-point within a few seconds and after cooling was centrifuged to remove the solid residue. Suitable aliquots, usually 10 μl., were used for chromatography.

Sugars. The paper chromatography of sugars has been described in detail by Partridge (1948). The solvents used in the present work were phenol, *s*-collidine, and a mixture of *n*-butanol 40 per cent., acetic acid 10 per cent., water 50 per cent. Reducing sugars were revealed by $AgNO_3$-ammonia. Other chromatograms were sprayed with the resorcinol-trichloracetic acid reagent described by Partridge. This reagent gives strong reddish brown spots with

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sugars containing ketoses, while pentoses yield grey-green spots. The trisaccharide raffinose reacts to give reddish brown spots. Some of the extracts investigated in the present work yielded similar spots which coincided with raffinose in R_F values in phenol and in butanol-acetic acid. Since the R_F values in the two solvents are widely different it is probable that the sugar of the plant materials is raffinose itself or some closely allied trisaccharide.

Organic acids. For the organic acids the method described by Lugg and Overell (1947, 1948) was adopted. In its present form the method appears to work only over a comparatively narrow range of concentrations, but although acids present only in traces will be overlooked the method is of considerable value in identifying the principal acids of a tissue. Thus, in a test using orange juice there was no difficulty in showing that malic and citric acids are present, with the latter at approximately 10 times the concentration of the former.

In all the work described the untreated water extract was used. In more intensive work with materials available in considerable quantity, it is probable that preliminary extractions or fractionations would be of advantage.

Most of the materials were taken from greenhouse plants during the period February–March and collection was made at 11 a.m. in each case.

The following materials were investigated:

Bryophyta

Hepaticae: *Conocephalum conicum*, thallus and carpocephala, *Marchantia polymorpha*, *Monoclea Forsteri*, *Pellia epiphylla*, *Fossombronia angulosa*, *Diplophyllum albicans*.

Musci: *Polytrichum commune*, *Catharinea undulata*.

Pteridophyta

Psilophytinae: *Psilotum triquetrum*. Lycopodinae: *Selaginella Kraussiana*, *S. Willdenovii*, *S. caulescens*, *Isoetes echinospora*. Equisetinae: *Equisetum scirpoides*, *E. robustum*, *E. trachyodon*, *E. hyemale*, *E. variegatum*.

Filicinae: Ophioglossales; *Ophioglossum vulgatum*—leaf and rhizome. Marattiales; *Angiopteris evecta*—leaf and prothallus. Filicales; Osmundaceae, *Osmunda regalis*—leaf and prothalli (haploid, diploid, and tetraploid), *O. javanica*—leaf, *Todea barbara*—leaf and prothallus, *Leptopteris superba*—leaf *L. hymenophylloides*—leaf. Schizaeaceae, *Lygodium scandens*, leaf. Marsileaceae, *Marsilea quadrifolia*—leaf. Hymenophyllaceae, *Trichomanes radicans*—leaf. *Hymenophyllum demissum*—leaf. Polypodiaceae, *Dennstaedtia cicutaria*—leaf, *Nephrolepis cordifolia*—leaf, *Pteris cretica*—leaf, *Blechnum spicant*—leaf, *Phyllitis Scolopendrium*—leaf, *Dryopteris aristata*—leaf, rhizome, prothallus. *D. filix-mas*—leaf, *Polystichum angulare*—leaf, *Polypodium aureum*—leaf, *P. vulgare*—leaf, rhizome, *Platycerium alicorne*—leaf.

RESULTS

Since there are no striking differences between the principal groups investigated, the individual results will not be tabulated but are available for reference.

Bryophyta. In the limited range of Hepaticae and Musci selected, sucrose was invariably found, sometimes in fairly high concentration (*Conocephalum*—approximately 2·5 per cent.). Glucose and fructose were present in most cases but in smaller amounts. 'Raffinose' was detected in *Pellia*, *Diplophyllum*, and *Catharinea*.

In no case were organic acids revealed in the extracts, although in the two mosses and in *Diplophyllum* the pH was notably low (3·8–4·2). Apart from an isolated reference to the presence of oxalate (Garjeanne, 1932), there is apparently no information on the organic acids of bryophytes, and it would be interesting to know whether the low pH is due to a weak buffering of the sap or to the presence of volatile acids which escape detection by the methods employed.

Pteridophyta. Sucrose, glucose, and fructose were prominent in all the pteridophytes investigated with the exception of *Selaginella Kraussiana* and *S. caulescens*. In these species the sucrose is possibly replaced by trehalose which was discovered by Anselmino and Gilg (1913) in *S. lepidophylla* and has since been obtained by Yamashita and Sato (1929) from twenty-one Japanese and Manchurian species of *Selaginella*. Trehalose would not be revealed by the methods used in the present work.

'Raffinose' was found in certain genera which are usually regarded as primitive, namely *Psilotum*, *Marattia*, *Osmunda*, *Todea*, and *Hymenophyllum*, but not in *Ophioglossum*; it is apparently absent in the Polypodiaceae. Its presence is particularly striking in the Osmundaceae, where each of the five species investigated gave a pronounced reaction. The significance of this occurrence can only be determined by more extensive investigations.

In the prothalli available, *Angiopteris*, *Osmunda*, and *Dryopteris*, the sugars corresponded to those in the sporophyte of the same species. In *Angiopteris* and *Osmunda* it was particularly striking that 'raffinose' was present in the prothalli as well as in the sporophyte. No striking differences were found between the haploid, diploid, and tetraploid *Osmunda* prothalli. As far as the writer is aware there is no previous information in the literature as to the sugars of fern prothalli. In fact, there have been few studies of the sugars of pteridophytes. In one of the few works on this subject, Bäsecke (1908) failed to find sucrose in the leaves of *Phyllitis Scolopendrium*, but the presence of this sugar in abundance is readily demonstrable by the methods used here.

The results of the organic acid investigation support the view of Wetzel (1938) that malic acid is the chief acid of the Pteridophyta. Malic acid was detected in *Ophioglossum*, *Angiopteris*, *Marsilea*, *Hymenophyllum*, *Phyllitis*, *Dryopteris*, and *Polypodium*. It was present in greater quantity in all species of *Equisetum*, but the highest concentrations were found in the leaves of *Isoetes* and *Platycerium*, where the concentration of malic acid is quite comparable with that of the typical acid succulent *Bryophyllum calycinum*. Unidentified acids were present in considerable amount in the leaves of *Osmunda regalis*, *Trichomanes radicans*, *Dennstaedtia cicutaria*, and *Nephrolepis cordifolia*.

Organic acids were not detected in any prothalli. On the whole it would appear that the Pteridophyta contain only small amounts of non-volatile organic acids.

I wish to thank Professor C. W. Wardlaw for his interest in this work and Mr. E. Ashby for supplying some of the scarcer plant materials.

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Demonstration of Compound Cilia in a fern Spermatozoid with the Electron Microscope

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WITH 5 PLATES

THE fibrillar construction of contractile protoplasm in organs ranging from cilia to striped and unstriped muscle has been suspected at least since the time of Engelmann (1881). An excellent early paper dealing with the subject will be found in Dellinger (1909), which, besides giving a good historical survey of results obtainable with the light microscope, includes an admirable photographic demonstration of many-stranded cilia in certain Protozoa and Flagellates obtained simply by applying pressure to live cells under a cover-slip. With regard to plant cilia, a fibrillar structure obtained by maceration has been found in a number of cases, notably Mühldorf (1930) on the spermatozoid of *Sphagnum*, Miduno (1934) on the liverwort *Dumortiera*, and Dracinschi (1930) on the fern *Athyrium* and (1932) on *Isoetes*, *Selaginella*, and other members of the Pteridophyta. In other cases, notably Yuasa (1938) and Manton (1950), a fibrillar structure was revealed without special maceration merely by killing fern spermatozoids with osmic vapour. These observations, however, constitute a qualitative demonstration only. The component strands, no matter how they are separated, are so slender that they cannot be adequately seen by any ordinary microscopic method even when their thickness has been enhanced by special methods of drying and staining. Indeed, the only instance known to us in which even the qualitative demonstration has been achieved without such staining methods is that of Manton (1950), in which the fibrils could be demonstrated in the wet condition and still attached to the body of the spermatozoid, by the use of ultraviolet microscopy. Any further progress, especially in a quantitative direction, must clearly await the application of more powerful methods than light microscopy.

The present note is an attempt to amplify the observation of Manton (1950) on the structure of the cilia of a fern spermatozoid, this time with the aid of the electron microscope. With the ultraviolet microscope the number of strands per cilium had been roughly assessed as 'of the order of ten', the species being a somewhat unusual one (a diploid form of *Dryopteris Villarsii* native to the island of Cyprus) which had happened to be available in quantity when material for the observation was required. The continued use of an exotic species for an observation which ought to be fundamental seemed, however, undesirable, and the micrographs reproduced here all refer to the Common Male Fern, the tetraploid species *D. Filix-mas* (L.) Schott, s. str., the spores of which were collected wild near Leeds and germinated for teaching purposes in the roof greenhouse of the Botany Department of the University.

The technical treatment of the specimen was as close to that used for the ultraviolet observation as the different requirements of the two instruments permit. A drop of water containing vigorously swimming spermatozoids of the fern (hatched out by merely submerging rinsed prothalli in tap-water) was placed on a prepared carrier covered with the appropriate formvar film, and immediately exposed to the vapour from 2 per cent. osmic acid for 30 seconds. Surplus liquid was then sucked off with filter-paper without touching the film itself and the specimens left to dry at room temperature. This takes only a few minutes, after which they were shadowed with uranium and examined.

Fig. 2 shows a general view of part of the front end of a spermatozoid bearing a large number of cilia in various stages of fibrillar unravelling. Some enlarged details of these are contained in Fig. 3, from which the fibrillar nature of both intact and unravelled cilia is clearly displayed. In the intact condition the fibrillar structure is revealed by longitudinal striations traversing the whole length of the cilium in a slightly spiral path. In the unravelled condition the component strands are widely splayed.

More precise details about individual cilia can be obtained from Figs. 1, 4, and 5, all of which refer to detached cilia lying loose upon the formvar film and not definitely referable to any particular body. In each of these figures, some of which are compounded from several different exposures owing to the extreme length of the organs depicted, one or more whole cilia are contained, together with portions of others. Various grades of unravelling are represented, certain cilia (Fig. 1) having varying lengths of their basal portions intact, while in other cases (Figs. 4 and 5) virtually the whole free length of the cilium shows its fibrillar nature.

An impressive fact about all these cases, and numerous others which collectively amount to some fifty separate cilia derived from several different cells, is their complete numerical uniformity. There are exactly eleven strands present in each, two of which are somewhat different from the others and appear to be centrally placed. These two central strands are easily distinguished in the dry condition by being thinner, shorter, and more contorted than the others, and they cohere somewhat more closely together.

These numerical facts can be very easily seen in the more highly magnified view of the distal end of a cilium contained in Fig. 7. This figure (in which the shadows appear white owing to the fact that it is a direct print) also shows particularly clearly the range of difference in length among the thicker fibrils to which the tapering point in the intact cilium is undoubtedly due. One of the fibrils in the figure has been broken where it is crossed by another, but the rest are entirely undamaged and very nearly in their original positions. These observations can be extended and confirmed by close scrutiny of Figs. 1, 4, and 5.

It is not yet possible to extend the description of the individual fibrils to include details of their finer structure, but it is probable that some further microstructure will eventually be detectable within each strand which at present is only imperfectly indicated by a few longitudinal lines visible under the highest magnifications used, e.g. Fig. 6. The dimensions of the strands in the dry condition can, however, be roughly assessed by comparison with standard granules of polystyrene[1] scattered onto the preparation after the shadowing was complete and therefore readily distinguished by their absence of shadow from natural granules liberated by the specimen. One of these is contained in Fig. 6, and in relation to it the diameter of the individual strands can be roughly assessed as of the order of 700 A (= 0·07 μ). This is a fairly large diameter which would admit of considerable complexity of internal structure, although it is well below the limit of true resolution with the light microscope, as was surmised at the outset.

The spiral configuration of the cilium as a whole is more strikingly revealed in the unravelled specimens than in the intact ones. A particularly clear example which scarcely admits of any other interpretation is the basal end of the cilium of Fig. 5. The number of turns in the whole cilium appears to be of the order of two in this cilium and not more than three in longer ones. This is a very steep spiral which may nevertheless be of considerable importance in determining the type of motion of the organ.

Fig. 5 is also important as a very clear example of an appearance which is most easily interpreted as denoting the presence of some intercalary substance between the strands and which when dried gives a rough or granular surface to them. The cleaner, smoother surfaces of the component strands of most of the other cilia depicted (cf. especially Fig. 7) is then most easily explained as being due to the more complete washing away of this material.

The presence of an intercalary adhesive substance uniting the component strands of a cilium in life and dissipated in varying degrees by the action of osmic vapour on killing is the most probable explanation of all these various appearances. Its chemical nature is unknown, but it is perhaps worth noting that the ease with which it can be dispersed by this simple chemical treatment varies very considerably from specimen to specimen for reasons which are at present undiagnosed. Sometimes, as on this and the previous occasion, the effect of osmic vapour is immediate and complete. On other occasions, with the same and other species the component strands of the cilia remain obstinately united under treatment which to all appearances has been identical. This is perhaps the reason why the compound nature of fern cilia has not been more fully demonstrated by other workers.

The conclusion seems inescapable that the clarity with which the ciliary strands are splayed out in this and the previous species (*D. Filix-mas* and *D. Villarsii*) is an artifact induced by the method of killing, and that in all species in life a cilium is effectively a single organ in which the component strands are stuck together by something more substantial than merely surface forces.

The electron microscope used for this work is the new Philips instrument

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[1] The use of polystyrene latex particles for purposes of measurement is described by Drummond (1950), p. 116. The diameter of these particles is of the order of 2,500 A with an error of 12 per cent. in the determination. This diameter is between three and four times that of a ciliary fibril; hence the rough estimate of 700 A for their diameter.

recently installed in the Botany Department of Leeds University with the aid of a grant from the D.S.I.R. We are grateful to Dr. R. D. Preston, for whose work it was primarily installed, for permitting us to use it. We are also indebted for financial assistance to the Agricultural Research Council.

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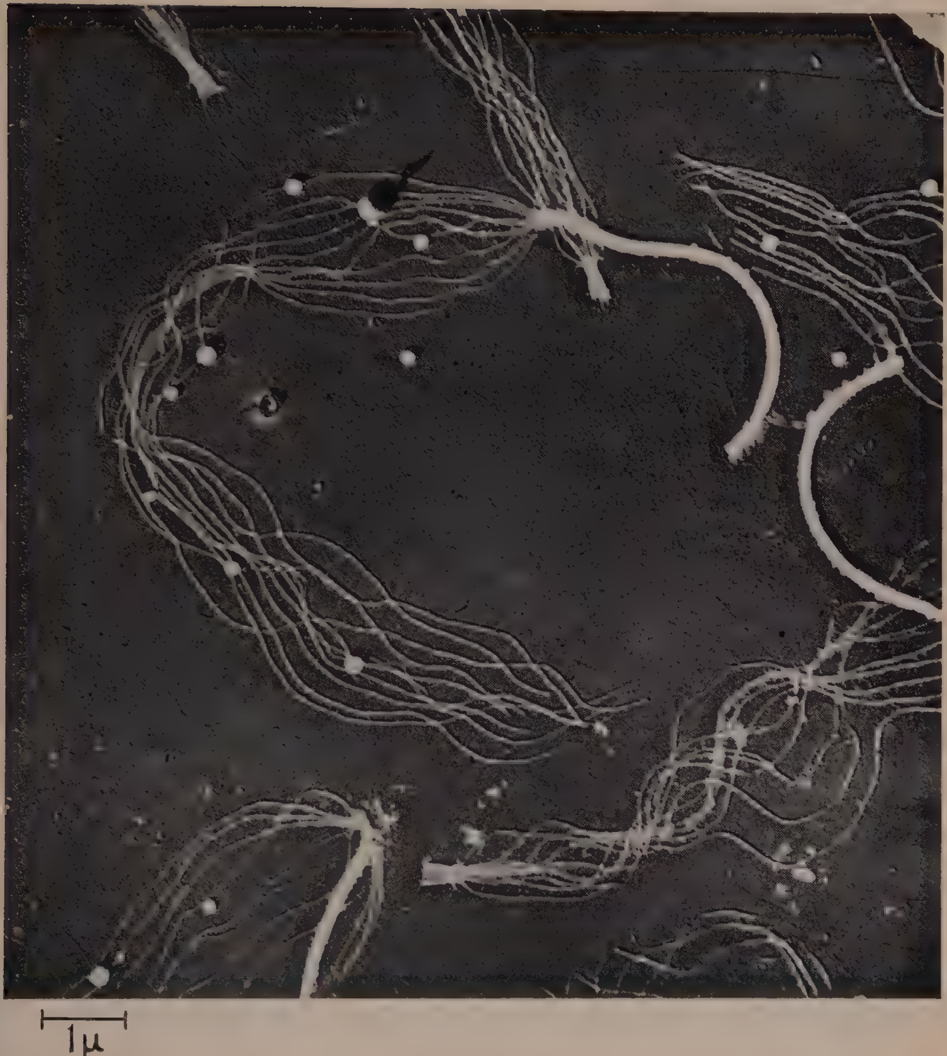


FIG. 1. Detached cilia of the Male Fern (*Dryopteris Filix-mas* (L.) Schott s. str.) showing various degrees of fibrillar unravelling. Electron micrograph No. M 17.13, uranium shadowing, 80 kV., magnification × 10,000.

I. MANTON AND B. CLARKE—PLATE I

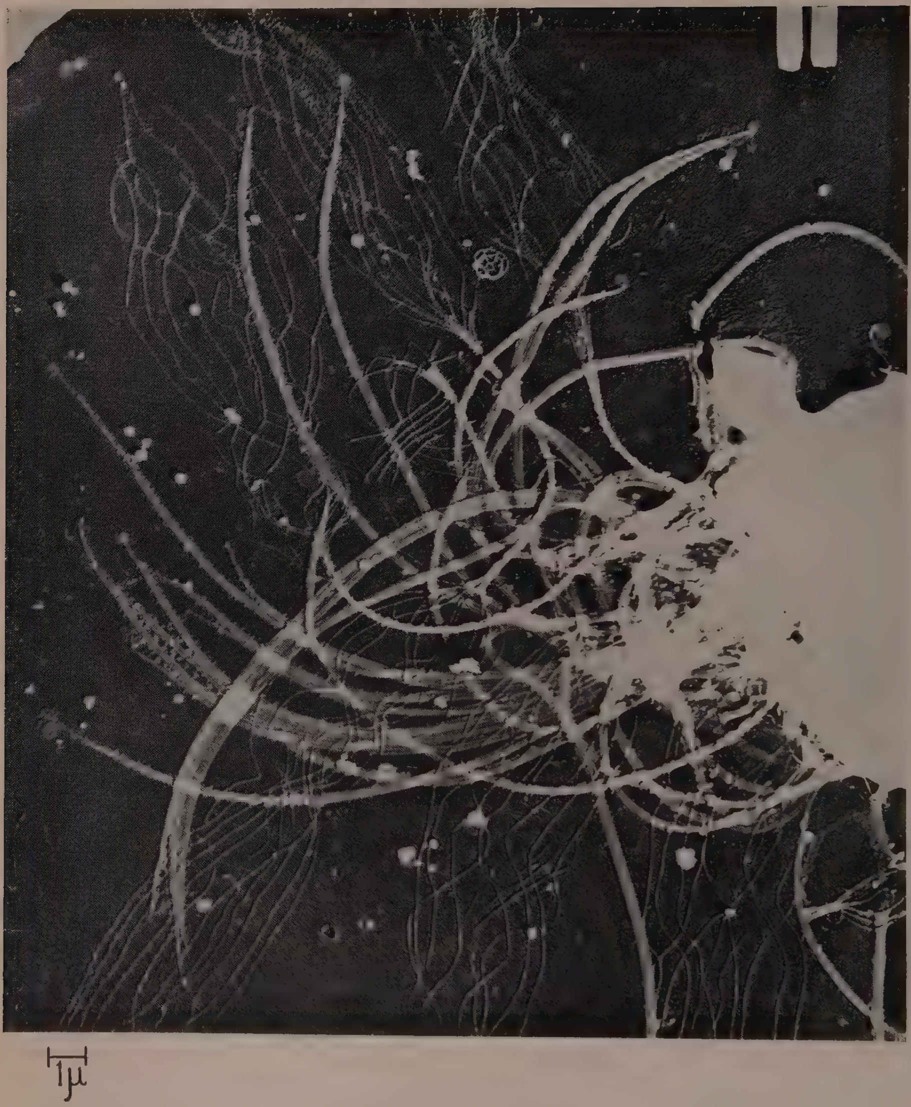


FIG. 2. Part of the front end of a spermatozoid of the Male Fern with attached cilia in various grades of fibrillar unravelling. Electron micrograph No. M 17.4, uranium shadowing, 80 kV., magnification × 5,000.

I. MANTON AND B. CLARKE—PLATE II

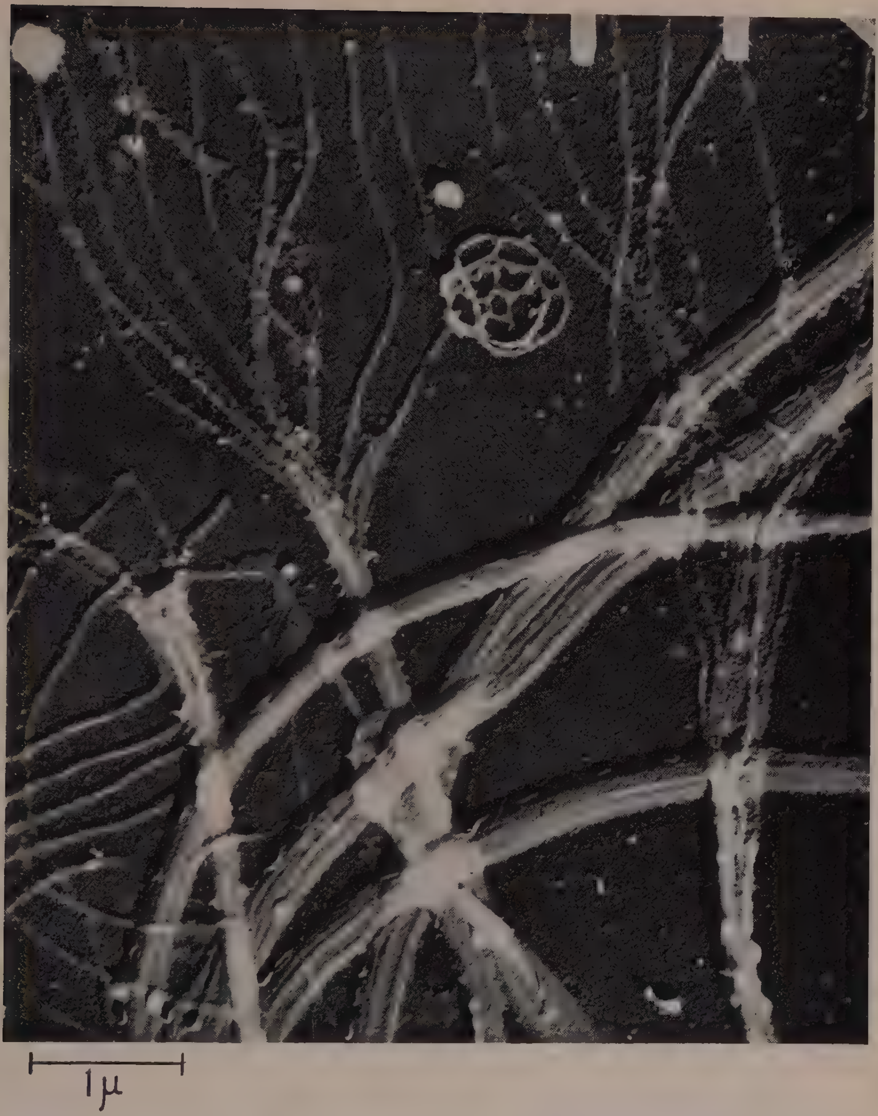
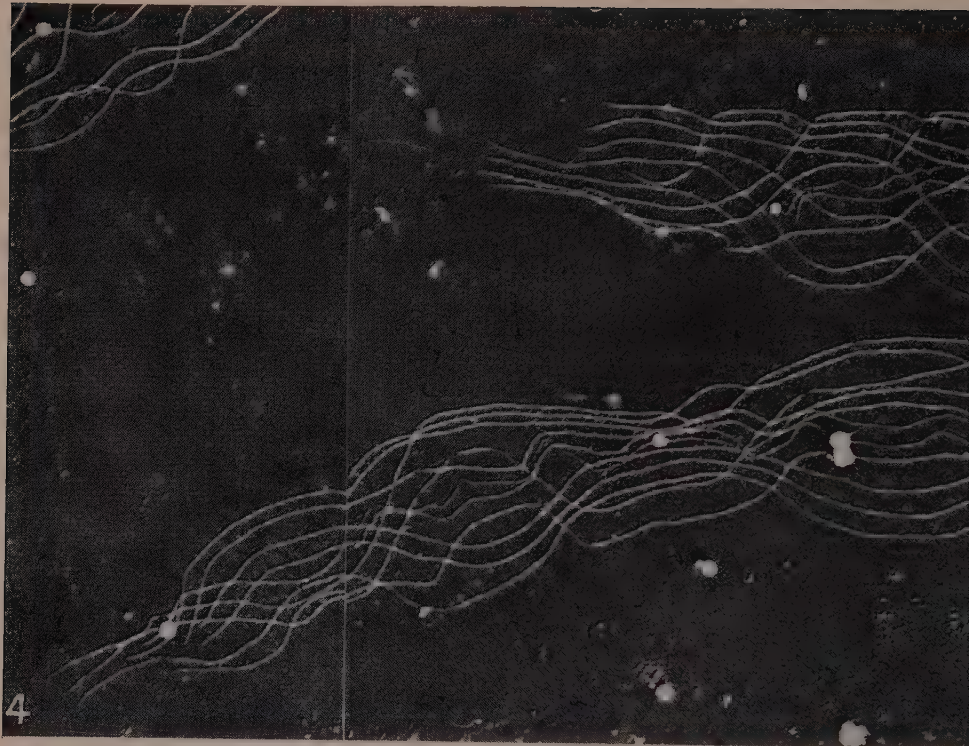


FIG. 3. Part of the field of Fig. 2, more highly magnified. Electron micrograph No. M 16.9, 60 kV., magnification ×20,000.

I. MANTON AND B. CLARKE—PLATE III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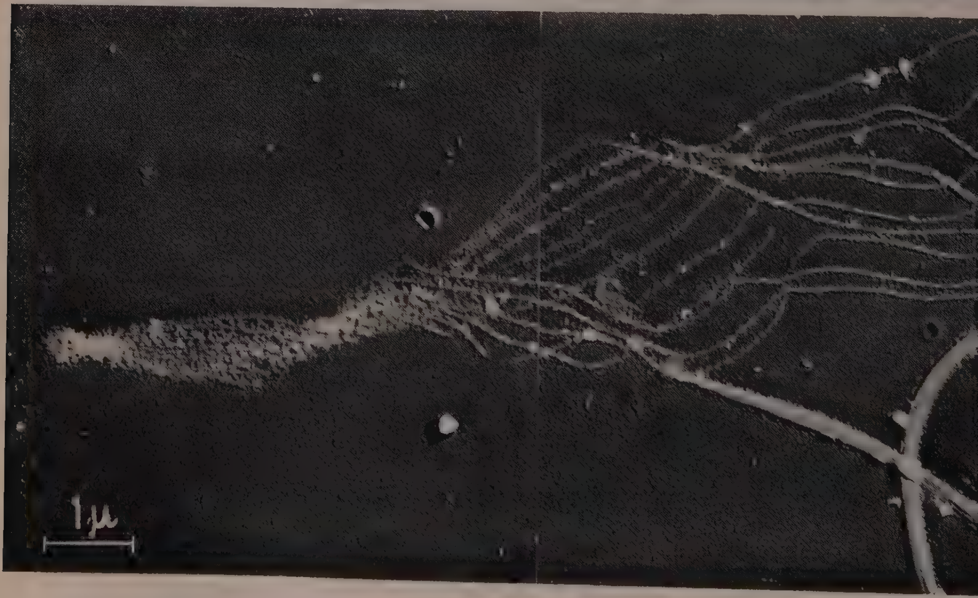
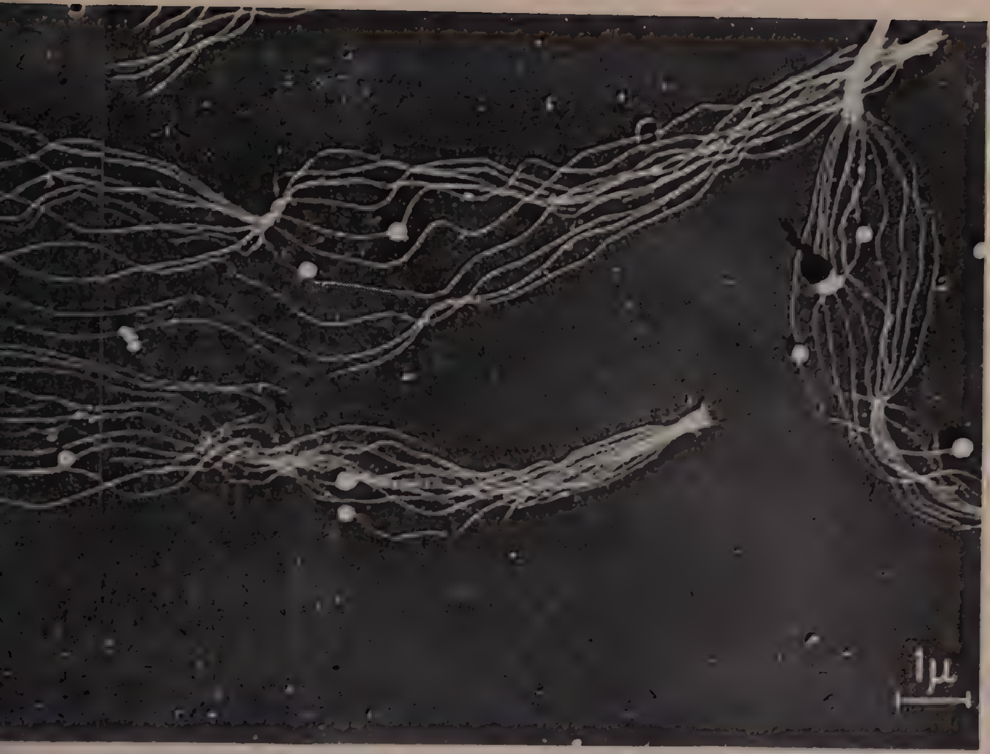
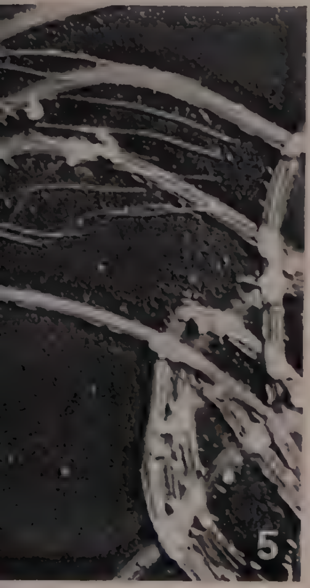
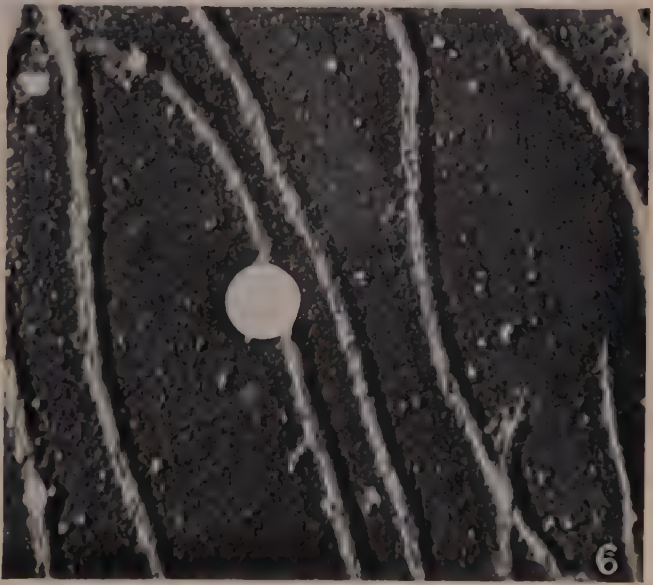


FIG. 4. Detached cilia more completely unravelled. Electron micrographs Nos. M 17.22–24,
FIG. 5. Detached cilium showing very clear spiral twist and traces of cementing substance be
FIG. 6. Small portion of an unravelled cilium with a polystyrene granule of known dimensions

I. MANTON A







ing, 80 kV., magnification ×10,000.

Electron micrographs Nos. M 17.7–8, uranium shadowing, 80 kV., magnification × 12,000.

ctron micrograph No. M 21.3, 60 kV., magnification ×40,000.

KE—PLATE IV

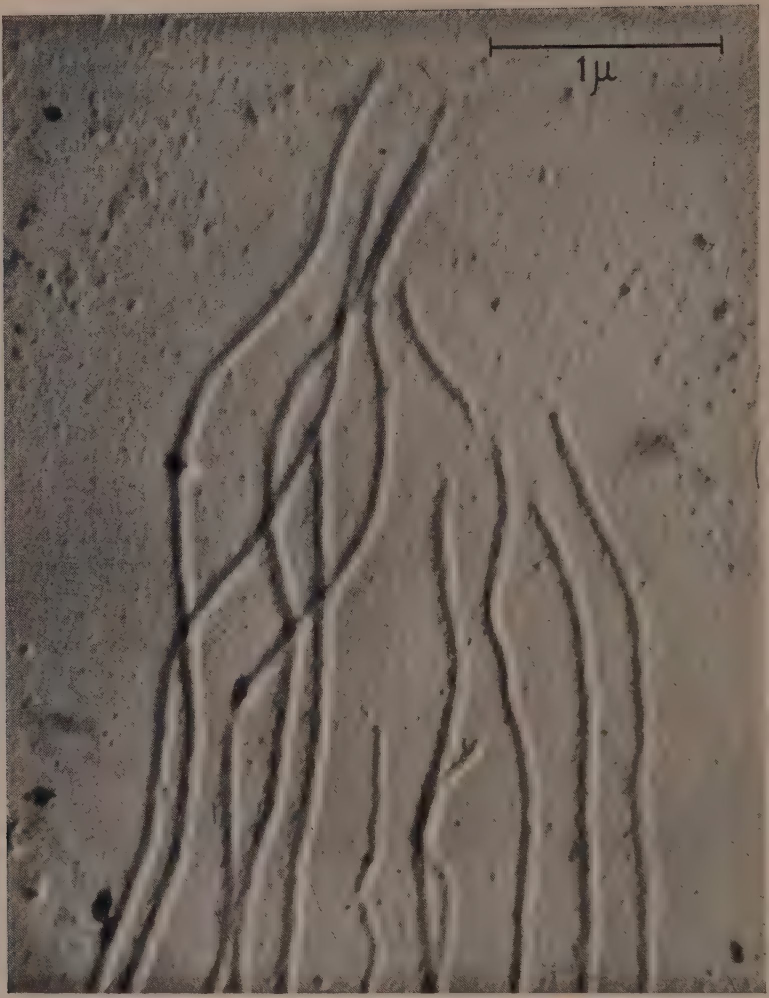


FIG. 7. Distal end of an unravelled cilium showing the two short central strands and nine thicker peripheral strands of slightly differing length. Direct print of electron micrograph No. M 21.10, uranium shadowing, 60 kV., magnification ×30,000.

I. MANTON AND B. CLARKE—PLATE V

The Water Permeability of Unplasmolysed Tissues

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SUMMARY

1. The permeability of unplasmolysed cells of beetroot, v. 'Crimson Globe', was determined from the rate of water loss of beet slices on placing in sucrose solutions having O.P. greater than the suction pressure of the beet. The absolute values obtained were about 0·7 μ^3 water per μ^2 cell-surface per hour per atmosphere osmotic pressure difference, i.e. 0·7 μ/hr./atm.

2. The permeability of similar beet cells plasmolysed within their cell walls was found to be about 13 μ/hr./atm.

3. The permeability of beet cells which had been plasmolysed and allowed to recover was shown to be approximately the same as that of unplasmolysed cells.

4. The hypothesis is advanced that the increase in water permeability on plasmolysis is due to those parts of the plasma-membrane which had formerly been pressed against the micelles of the cell wall becoming free and able to take part in water transfer.

5. The energy requirement for the maintenance of an excess hydrostatic pressure of five atmospheres within a cell by its vital activity was shown to be about one-tenth of the total respiratory energy released in freshly cut beet slices.

1. INTRODUCTION

PAST determinations of the absolute value of the permeability of plant cells to water have for the most part been made on protoplasts out of contact with their cell walls. Levitt, Scarth, and Gibbs (1936) used protoplasts isolated from the pulp cells of onion scales as experimental material: Resuhr (1935) used unfertilized eggs of *Fucus*. Others have used protoplasts *in situ* which, for convenience in measurement, have been plasmolysed. Huber and Höfler (1930) used cells of *Salvinia* and various algae; De Haan (1935), onion scale epidermis cells.

The results obtained by these writers are summarized in Table I.

TABLE I

Experimental material	Medium	Permeability μ/hr./atm.*	Measured by
Freed onion protoplasts	NaCl/CaCl$_2$ solutions	20	Levitt, Scarth, and Gibbs (1936)
Fucus eggs . . .	Sea-water sucrose solutions	10–20	Resuhr (1935)
Onion epidermis . .	Sucrose solution	30	De Haan (1931)
Salvinia leaf . .	KNO$_3$ solution	33	Huber and Höfler (1930)

* i.e. the speed of an advancing front in μ per hour under a pressure difference of 1 atmosphere.

These results agree fairly well among themselves and also with the permeabilities of animal cells, collated by Lucke, Hartline, and Ricca (1939).

Huber and Höfler, in calculating the water permeability of *Salvinia*, assumed that those parts of the protoplast which were in contact with the cellulose cell wall were out of action in water transport or at least had their permeability very much reduced. An indication of the validity of this assumption is given by the order of magnitude of their results which accord fairly well with those for naked protoplasts. Further, Huber and Höfler found that the two unequal halves of a cylindrical protoplast of *Salvinia* which had broken in two during plasmolysis shrank at the same rate. This they attributed to the fact that loss of water was only taking place through those parts of the protoplast halves which were free of the cell wall, i.e. the hemispherical ends, which had the same area in each part.

It might be expected that determinations of the permeability of unplasmolysed cells to water would give results much lower than those given in Table I. Such determinations have been made by Baptiste (1935) and by Aykin (1946). Neither of these sets of determinations are comparable with those of Table I since the results are expressed in terms of surface area of an arbitrarily chosen tissue disk: 0·5 mm. thick × 1·8 mm. diameter (Baptiste) and 10 mm. thick × 8 mm. diameter (Aykin). The actual cell-surface area presented by such tissue disks to an external medium is much greater than the superficial area of the disks, due to the curvature of the surfaces of the exposed cells, even if penetration of the disks by the medium is negligible. The comparative value of these results with others in the work in which they appear is not questioned: but they cannot be considered absolute measures of the permeability of unplasmolysed plant cells.

In the present work the primary aim has been to determine the relative and absolute magnitudes of the permeability of a plant tissue to water in the plasmolysed and unplasmolysed states. Root parenchyma of *Beta vulgaris* (red varieties) was used as experimental material.

2. THE DETERMINATION OF THE WATER PERMEABILITY OF UNPLASMOLYSED CELLS

(*a*) *Experimental methods*

The water permeability of a cell or tissue is satisfactorily expressed by the formula

$$P_w = \frac{W}{A \times \text{M.P.D.} \times t},$$

where P_w = permeability of the cell to water,
W = volume of water lost or gained in time t,
M.P.D. = mean pressure difference impelling water into or from the cell,
A = area of cell-surface.

The methods used to evaluate each of these terms will be discussed below.

It is not possible to derive W from measurements of the linear dimensions of a cell when it is unplasmolysed. W was therefore determined from the gross change in weight of tissue slices when placed in solutions of varying osmotic pressure. In order to obtain consistent results in weighing, the slices were dried on removal from the medium, between filter-paper, under a weight of 50 g., for a standard 5-second period. This procedure is that used by Ashby and Wolf (1947). The slices were weighed in a stoppered vessel.

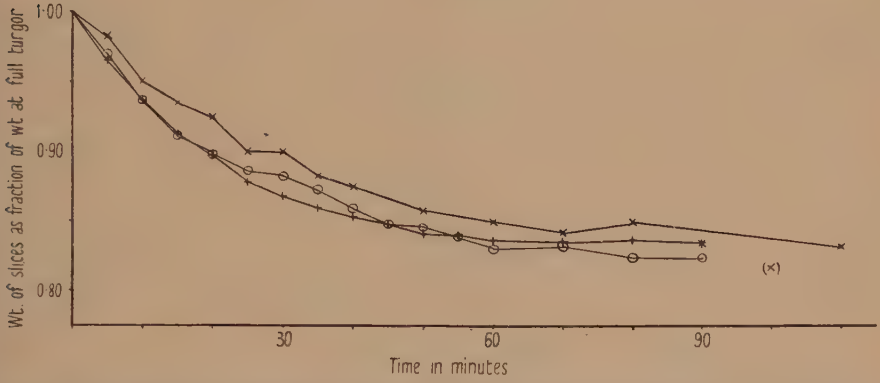

FIG. 1. Time course of water loss from fully turgid beet slices on placing in 12-atm. sucrose solution. Slice-thickness, 0·78 mm. × ; 0·39 mm. o; 0·185 mm. +

In selecting a suitable slice-thickness the fraction of cells damaged by cutting must be kept as low as possible without reducing the rate at which the innermost cells come into equilibrium with the medium. Fig. 1 shows the course of water loss from slices 0·78, 0·39, and 0·185 mm. thick when placed in 12-atm. sucrose after equilibration with aerated distilled water.

It will be seen that reduction of the slice-thickness from 0·39 mm. to 0·185 mm. does not result in an appreciable increase in the rate of water loss. Therefore the innermost cells of a slice 0·39 mm. thick must be losing water at the same rate as those of the thinner slice. This can only be the case if the rate of loss of water is limited by the cell itself and not by the resistance of the paths communicating with the external solution. Therefore at a pressure difference of 12 atm. or below the cells of a slice 0·39 mm. thick behave as if each was bathed in the external medium. Further evidence on this point may be given. If a tissue slice in which the resistance to the passage of water is high is considered, it will be seen that the outer cells would come into equilibrium with a hypertonic medium first. Towards the end of the equilibration period only the innermost cells would be losing water and this water would have to reach the outside via a high-resistance route. The value of P_w would therefore be lower than at the outset. Reference to Table IV will show that no such fall is shown by slices of the thickness used and that the innermost cells are therefore not to any extent lagging behind the outer cells in coming into equilibrium with the medium.

Since, as has already been stated, the cells behave as if each was bathed in the external medium, the effective surface area, A, will be the sum of the areas of all the cells. This is conveniently expressed as the Specific Area—the cell-surface area per gramme of tissue.

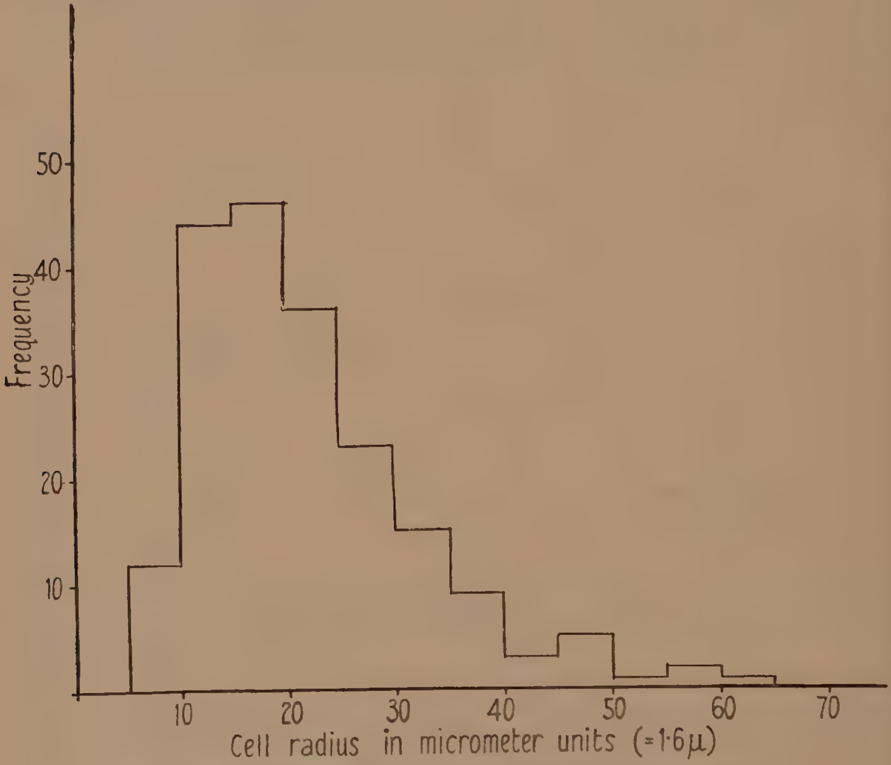

FIG. 2. Size-distribution of cell radii in beetroot 'Crimson Globe'.

In calculating the specific area it is assumed that the cells are closely packed spheres of unit density: the error of area introduced by regarding the cells as spheres is offset to some extent by the error of weight due to water in the intercellular spaces. The cells of the beet variety 'Crimson Globe', which was used in the present part of the work, are, in fact, very nearly spherical and a distribution curve of radii measured in all three mutually perpendicular planes shows no departure from unimodality as shown by the data plotted in Fig. 2.

The formula for obtaining A, the specific area, is derived as follows:

Let r = the radius of a sphere,
m = its mean value,
μ_2, μ_3 = the 2nd and 3rd moments about the mean of a distribution of values of r.

Then $$\text{Mean value of } r \left[= \frac{\sum r}{n}\right] = m,$$

$$\text{Mean value of } r^2 \left[= \frac{\sum r^2}{n}\right] = m^2+\mu_2,$$

$$\text{Mean value of } r^3 \left[= \frac{\sum r^3}{n}\right] = m^3+3m\mu_2+\mu_3.$$

Whence

$$A = \frac{\text{Area of } n \text{ spheres}}{\text{Volume of } n \text{ spheres}}$$

$$= \frac{4\pi(m^2+\mu_2)n}{\frac{4}{3}\pi(m^3+3m\mu_2+\mu_3)n}$$

$$= \frac{3(m^2+\mu_2)}{m^3+3m\mu_2+\mu_3},$$

or alternatively $$A = \frac{3(m^2+\sigma^2)}{m^3+3m\sigma^2+\sigma^3\gamma_3},$$

where $\sigma =$ standard deviation ($\sqrt{\mu_2}$),

and $\gamma_3 =$ 3rd measure of skewness ($= \mu_3/\sigma^3$).

Table II shows the value of A for seven different distributions. Each was calculated from the radii of 200 cells.

TABLE II

Determination of specific area

Unit	Mean radius	Standard deviation	γ_3	Specific area in cm.^{2} per cm.^{3}
(*a*) V. 'Crimson Globe'.				
8.5×10^{-4} cm.	4·05	2·9	+0·496	485
8.5×10^{-4} cm.	4·41	1·74	+3·86	545
1.65×10^{-4} cm.	21·58	11·2	+0·96	555
1.65×10^{-4} cm.	20·75	7·35	+0·59	700
			Mean value	*571*
(*b*) Another (purchased) variety				
1.65×10^{-4} cm.	27·53	12·3	+1·055	479
1.65×10^{-4} cm.	30·6	13·8	+0·97	430
1.65×10^{-4} cm.	28·3	12·4	+0·903	474
			Mean value	*460*

The mean pressure difference between the tissue and the external solution remains to be determined. The initial suction pressure of the tissue was adjusted to zero by equilibration with water, and thus the initial pressure difference was known. The intermediate suction pressures were determined by the refractometric method of Maximov, described by Ashby and Wolf (1947). In this method the change in concentration of a series of sucrose solutions when tissue slices are placed in them is measured refractometrically.

A tissue slice placed in sucrose solution of osmotic pressure equal to its suction pressure neither gains nor loses water: the concentration of the solution thus remains constant, whilst those of higher or lower osmotic pressure fall or rise

Data

Initial R.I. .	1·3335	1·3395	1·3465	1·3491	1·3519	1·3572	1·3650
O.P. . . .	0·0	3·0	6·7	8·0	9·5	12·2	17·0
R.I. change ($\times 10^4$)	+2	+4	+2	+1	−4	−11	−28

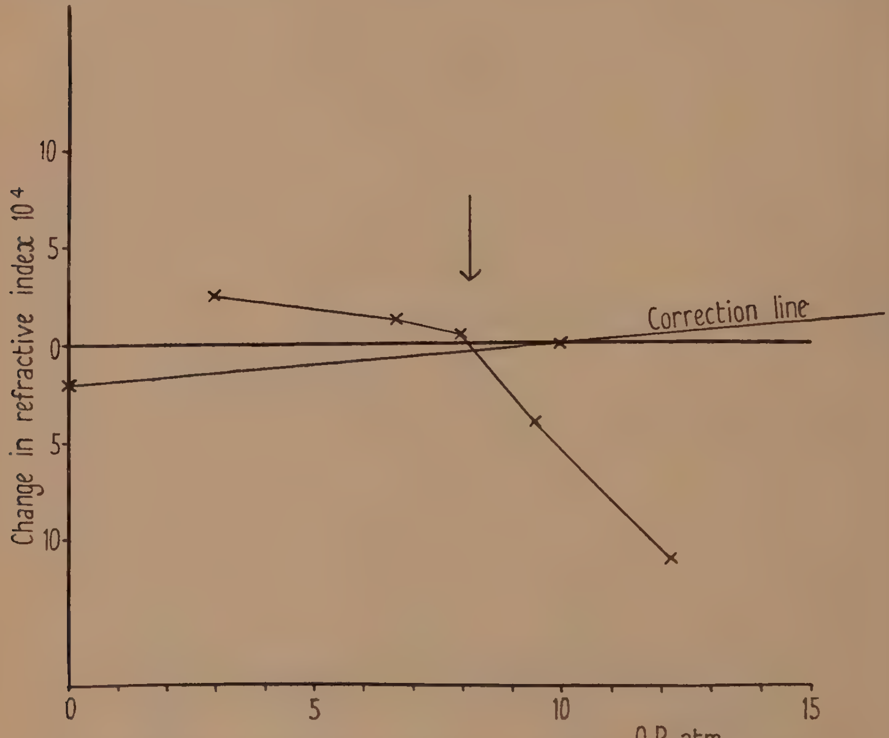

FIG. 3. Specimen suction pressure determination showing S.P. of tissue = 8·2 atm.

respectively. This method is rather insensitive at low-suction pressures since refractive index bears a hyperbolic relation to the volume of a solution containing a fixed amount of solute. Nevertheless, satisfactory results were obtained using 100-mg. tissue slices in 1 ml. of solution, using an Abbé refractometer reading to ±0·0001.

After drying by the standard method the tissue slices to be used for suction-pressure determinations still contain some sucrose solution transferred from the medium in which they have previously been. This causes an error in suction-pressure determinations. If a series of tissue slices removed from 10-atm. sucrose solution are placed in a series of solutions for suction-pressure determination, it will be seen that the change in refractive index due to solution transference will be zero in the 10-atm. member of the set and will increase steadily until in the zero atmospheres member the error will be at a maximum, and any water uptake by the cells of the tissue will have a negligible effect. In pure water, of course, a tissue slice, however high its suction pressure, produces no change of refractive index. The refractive index changes in solutions of suction pressures between 0 and 10 atm. may therefore be corrected for the solution transference error by referring the change to a correction line, joining zero refractive index change at 10 atm. and a change equal but opposite in sign to that in distilled water at zero atmospheres.

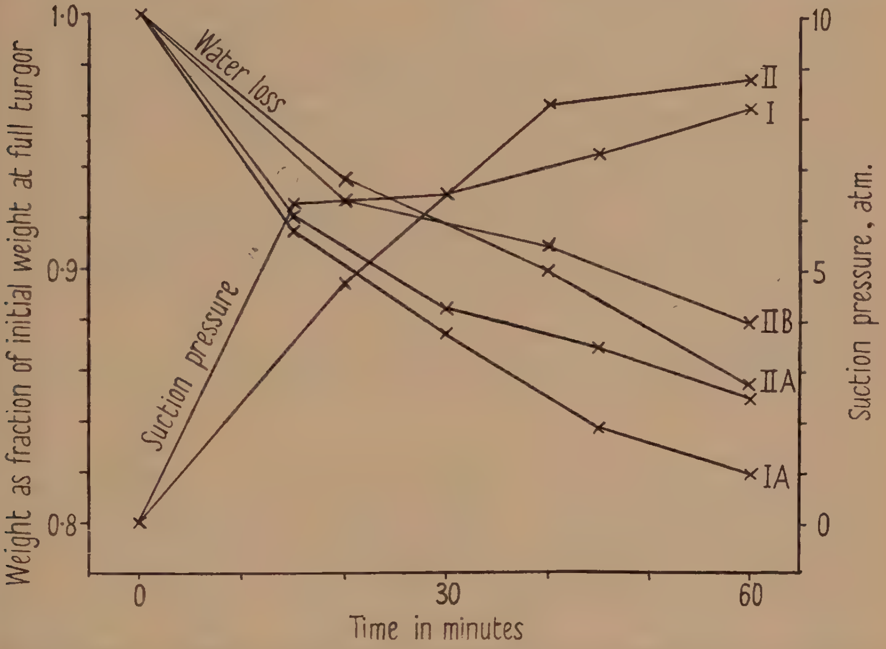

FIG. 4. Course of water loss and suction pressure rise in fully turgid beet slices on placing in 9·5-atm. sucrose solution.

This is illustrated in Fig. 3. The mean pressure difference was taken as being $P_e - \left(\frac{S - S_1}{2}\right)$ where P_e = O.P. of the external solution and S and S_1 the suction pressure of the tissue at the beginning and end of the time interval.

The experimental procedure was as follows. Beet slices 0·39 mm. thick and about 1·5 cm. square, weighing about 100 mg., were cut on a hand microtome, washed in several changes of glass distilled water to remove cut-cell debris, and placed in distilled water, which was kept stirred and aerated by a stream of air overnight. In the morning the water was replaced by fresh distilled water and aeration continued for 1–2 hours. At the end of this time the suction pressure of the slices was zero. The slices were removed in pairs, dried by the standard procedure, and weighed. They were then placed in beakers containing 20–25 ml. of sucrose solution of accurately known osmotic pressure, about

10 atm. The beakers were shaken mechanically to ensure aeration and prevention of local dilution of the solution by exosmosing water. At intervals of 15 or 20 minutes the slices were removed in pairs as before, dried, weighed, and replaced in the solution.

Similarly treated unweighed slices in sets of six were used for suction-pressure determinations, being removed a set at a time from a separate beaker of sucrose solution, dried, and placed in the series of sucrose solutions for suction-pressure determination. The ratio of the number of tissue slices to the volume of sucrose solution in the beakers was the same for those slices which were to be weighed as for those used for suction-pressure determinations.

The values of the osmotic pressure of sucrose solutions of known concentrations were taken from Berkeley and Hartley (1916): the refractive indices of sucrose solutions from U.S. Department of Commerce National Bureau of Standards Circular C 440.

(*b*) *Results*

Results are given below in tabular form and are also presented in graphical form in Fig. 4.

TABLE III

Suction pressures and fractional weights of slices of beetroot parenchyma (v. 'Crimson Globe') equilibrated in distilled water and transferred to 9·5 atm. sucrose at 0 minutes

Series I

Time (min.)	S.P. (atm.)	Weight slice *A* (mg.)	Fractional weight of *A* $[W/W_0]$	Weight slice *B* (mg.)	Fractional weight of *B* $[W/W_0]$
0	0·0	242·5 (= W_0)	1·00	235·2 (= W_0)	1·00
15	6·3	221·7	0·915	217·0	0·921
30	6·5	212·0	0·875	208·5	0·885
45	7·3	203·2	0·838	204·8	0·870
60	8·2	199·2	0·820	194·7	0·849
180	(9·5)	197·0	0·811	192·2	0·819

Series II

Time (min.)	S.P. (atm.)	Weight slice *A* (mg.)	Fractional weight of *A* $[W/W_0]$	Weight slice *B* (mg.)	Fractional weight of *B* $[W/W_0]$
0	0·0	243·7 (= W_0)	1·00	240·7 (= W_0)	1·00
20	4·75	228·0	0·935	222·2	0·927
40	8·25	219·4	0·90	219·2	0·91
60	8·75	208·0	0·855	213·7	0·88
180	(9·5)	204·2	0·840	207·7	0·86

TABLE IV

Permeability to water in μ/hr./atm. calculated from the data of Table III

Series I			Series II		
Time	*A*	*B*	Time	*A*	*B*
0–15	0·88	0·82	0–20	0·46	0·52
15–30	0·85	0·77	20–40	0·57	0·27
30–45	0·93	0·39	40–60	2·2	1·5
45–60	0·95	1·15			

The increase in permeability shown in Series II towards the end of the experiment is almost certainly due to experimental error. The mean value of the permeability of beetroot parenchyma to water is approximately 0·7 μ/hr./atm.

3. THE DETERMINATION OF THE WATER PERMEABILITY OF PLASMOLYSED CELLS

Methods

Since the values obtained for the permeability of unplasmolysed cells of beetroot are so much smaller than those of freed or plasmolysed protoplasts of onion, &c., given in Table I, it is of interest to determine the water permeability of beet protoplasts plasmolysed within their cell walls.

Beet slices about 100 μ thick were plasmolysed in 21-atm. sucrose solution for an hour. At the end of that time many of the protoplasts had assumed a spherical shape and were free of their cell walls. Clearly the protoplasts were touching the cell wall in places, but this is rather a different phenomenon from the contact of wall with protoplast where the pressure may be of the order of 12–18 atm.

A suitable section was mounted in the plasmolyticum on a chambered slide. A cell showing spherical plasmolysis was selected and measured with a calibrated micrometer eyepiece. The amount was then irrigated with 35-atm. sucrose solution and the course of contraction of the cell followed, measuring at half-minute or minute intervals until no further change was recorded. The osmotic pressure of the internal solution resulting from irrigation of the mount was calculated by assuming the volume of the cell to be inversely proportional to the internal osmotic pressure. In this way the internal osmotic pressure of the cell could be assessed and, at equilibrium, the osmotic pressure of the external medium.

The error introduced by this assumption is not large. Table V shows the values of PV for a sucrose solution (in which departure from $PV =$ constant is far more marked than in solutes with smaller molecules, which make up a good part of the cell-sap) and for onion protoplasts, measured by Levitt, Scarth, and Gibbs (1936).

TABLE V

O.P. (atm.)	PV (Onion cell)*	PV (sucrose solution)
18·4	1·0	1·0
27·4	1·09	1·12
36·4	1·07	1·22

The error introduced by assuming the cell-sap to be an ideal solution ($PV =$ constant) is less than that which would result from treating it as a sucrose solution. The maximum error in the pressure range used is under 10 per cent.

* Data of Levitt, Scarth, and Gibbs (1936).

The permeability to water of plasmolysed cells ($P_{w\text{ plasm.}}$) was calculated from the following formula:

$$P_w = \frac{\text{Volume change}}{\text{Average area} \times \text{M.P.D.} \times \text{time}}$$

$$= \frac{\frac{4}{3}\pi(r^3 - r_1^3)}{4\pi\frac{(r^2+r_1^2)}{2} \times \text{M.P.D.} \times t} = \frac{\frac{2}{3}(r^3 - r_1^3)}{(r^2+r_1^2) \times \text{M.P.D.} \times t},$$

where r, r_1 = radii of the cell at beginning and end of the experimental time interval, t = time interval, M.P.D. = mean pressure difference $= P_e - \left(\frac{P+P_1}{2}\right)$ where P_e = external O.P. and P and P_1 = internal O.P. at beginning and end of the time interval.

Since the changes in cell diameter were measured directly and not from a photograph or projected image (Levitt, Scarth, and Gibbs, 1936; De Haan, 1931), a high degree of accuracy cannot be attained by this method, especially as equilibrium is approached and the rate of shrinkage becomes slower. Nevertheless, a clear indication of the order of the permeability is given (Table VI).

The mean value of $P_{w\text{ plasm.}}$ is therefore about 13μ/hr./atm., the range (some of which is due to experimental error) being from 6–20.

4. THE PERMEABILITY OF CELLS RECOVERED FROM PLASMOLYSIS

(*a*) *Experimental methods*

With the object of investigating the recorded difference between the permeability to water ($P_{w\text{ plasm.}}$) of plasmolysed beet cells and that of unplasmolysed cells ($P_{w\text{ unplasm.}}$), the permeability to water of cells which had been plasmolysed and allowed to recover was determined.

Tissue slices which have been plasmolysed in 25-atm. sucrose solution fail to recover if the tissue is transferred to distilled water directly. The effect appears to be a purely mechanical one, the cells expanding so rapidly that the membrane is disrupted with consequent loss of the coloured cell-sap. By diluting the plasmolyticum slowly over a period of 2 hours, eventually transferring to aerated distilled water for 16 hours, tissue slices were obtained in which only a small percentage of the cells were damaged. The permeability of these slices was determined by the method described previously.

(*b*) *Results*. These are given in tabular form (Table VII).

The tissue which has been plasmolysed and allowed to recover has a permeability of the same order as the untreated tissue. The lower values obtained for the former are not considered significant—they are probably due to the fact that the damaged and empty cells have not only failed to lose water when placed in 9·5-atm. sucrose, but have actually gained in weight slightly by the replacement of water (density = 1·0) by the sucrose solution (density = 1·03).

TABLE VI

These results are shown graphically in Fig. 5.

Cell I (19/11)

Time	Radius (μ)	O.P.	$P_{w\ \text{plasm.}}$
0	21·0	20·6	
			6·2
½	20·6	21·35	
			16·2
1	20·2	23·1	
			20·3
1½	19·7	24·5	
2	19·4	26·1	
2½	19·4	26·1	
3	—	—	
Equil.	19·4	26·1	

Cell II (19/11)

Time	Radius (μ)	O.P.	$P_{w\ \text{plasm.}}$
0	17·8	20·6	
			12·6
½	16·6	25·4	
			5·72
1	16·1	27·1	
			13·5
1½	15·6	30·6	
			9·75
2	15·35	32·0	
2½	15·0	34·4	
3	15·0	34·4	
Equil.	15·0	34·4	

Cell III (19/11)

Time	Radius (μ)	O.P.	$P_{w\ \text{plasm.}}$
0	31·9	20·6	
			6·9
1	31·5	21·3	
			12·3
2	31·0	22·4	
			13·9
3	30·65	23·1	
			11·2
4	30·5	23·5	
			15·8
5	30·4	23·8	
6	30·2	24·2	
7	30·2	24·2	
Equil.	30·2	24·2	

Cell IV (19/11)

Time	Radius (μ)	O.P.	$P_{w\ \text{plasm.}}$
0	40·4	20·6	
			13·2
1	38·0	25·5	
			19·9
2	35·5	31·15	
3	33·9	35·9	
4	33·9	35·9	
5	33·9	35·9	
Equil.	33·9	35·9	

Cell V (21/11)

Time	Radius (μ)	O.P.	$P_{w\ \text{plasm.}}$
0	33·1	20·6	
			21·6
1	31·85	23·2	
			10·9
2	31·5	23·95	
			16·1
3	31·15	24·9	
4	31·15	24·9	
Equil.	30·85	24·9	

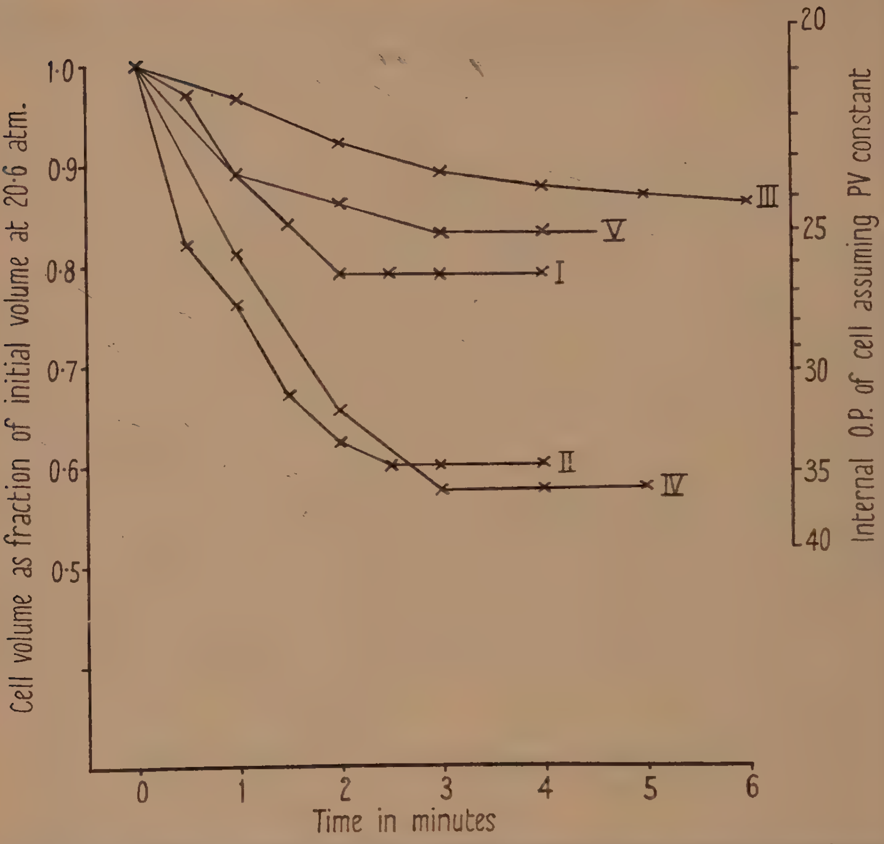

FIG. 5. Course of water loss from beet cells equilibrated with 20·6-atm. sucrose on transference to a stronger solution of O.P. in Expt. I, 26·1 atm.; II, 34·4 atm.; III, 24·2 atm.; IV, 35·9 atm.; V, 24·9 atm.

TABLE VII

Series I. Beetroot parenchyma recovered from plasmolysis. Transferred to 9·5 atm. sucrose at 0 minutes.

Time (min.)	S.P. atm.	Fractional weight (*A*)	Fractional weight (*B*)
0	0·0	1·00	1·00
30	8·9	0·924	0·945
120	9·5	0·85	0·90
	P_w (μ/hr./atm.) =	0·5	0·36

Series II. Controls. Unplasmolysed. Transferred to 9·5 atm. sucrose at 0 minutes.

Time (min.)	S.P. (atm.)	Fractional weight (*A*)	Fractional weight (*B*)
0	0·0	1·0	1·0
30	8·9	0·86	0·889
120	9·5	0·838	0·858
	P_w (μ/hr./atm.) =	0·92	0·72

5. DISCUSSION

I. From the results obtained in this investigation it will be seen that when the cell is turgid, its permeability to water is only about 5 per cent. of its permeability in the plasmolysed state, the actual values being approximately

$$P_{w\ \mathrm{unplasmolysed}} = 0{\cdot}7\ \mu/\mathrm{hr./atm.}$$

$$P_{w\ \mathrm{plasmolysed}} = 13\ \mu/\mathrm{hr./atm.}$$

The value of $P_{w\ \mathrm{plasmolysed}}$ approximates to those obtained by other workers with naked or plasmolysed protoplasts and to those obtained with a wide range of animal cells; *vide* section 1. This fact, together with the evidence of the present work, that a cell plasmolysed and exhibiting a water permeability of 10–20 μ/hr./atm. is capable of recovery, when its water permeability regains its normal 'unplasmolysed' value, indicates that the 'plasmolysed' value of P_w should be considered a characteristic of a plasmolysed but otherwise healthy cell. The failure of some cells to recover from plasmolysis, noted in section 4, can be controlled by adjustment of the conditions of deplasmolysis and is not therefore due to damage suffered by the cell during plasmolysis. The increase in water permeability on plasmolysis does not appear therefore to be due merely to disorganization of the cell as a result of shrinkage, and the best approach would appear to be to look for some factor limiting the rate of entry of water when the cell is turgid.

The simplest hypothesis which fits the facts is that the effective area of cell-surface is reduced by contact with the cell wall. The pressure forcing the protoplast against the cell wall at full turgor may be in the range 12–22 atm. in beet, and it seems probable that under pressures only a fraction of this, water transfer will be limited to those parts of the cell-surface which are apposed to pores in the cellulose wall. If, as has been generally assumed (e.g. Davson and Danielli, 1943), the cell-surface ('plasma membrane') is the principal resistance offered to the passage of water in and out of the cell, this reduction in effective area will lower the water permeability of the cell.

The volume of the interstitial spaces in natural cellulose fibres may be calculated from density determinations. Hermans (1946) showed from his own measurements and those of Frey-Wyssling and Speich (1942) that the density of natural and regenerated cellulose fibres measured by displacement in carbon tetrachloride is 1·55 g. per c.c., whilst if the density is calculated using the volume measured under the microscope, natural fibres (in this case the wall of the Ramie fibre) show a density of 1·39. Regenerated fibres retain the higher value. This discrepancy is due to the presence of a system of pores in natural fibres, which are penetrated by organic solvents and, it is inferred, by water. Measurements of the density made in water show other variations due to imbibition and allied phenomena.

The pore volume and the expected pore area in a surface can thus be calculated:

$$V_p = V_T - V_c = 1 - \frac{1\cdot 39}{1\cdot 55} = 0.103$$

$$A_p = A_T - A_c = 1 - \left[\frac{1\cdot 39}{1\cdot 55}\right]^{\frac{2}{3}} = 0.07,$$

where A_p, A_c, and A_1 are the pore, micelle, and total areas and V_p, V_c, and V_1 the corresponding volumes.

If the hypothesis given above is correct, the water permeability of an unplasmolysed cell should be about 7 per cent. of its plasmolysed value. The measurements made in this work show $P_{w\ \mathrm{unplasmolysed}}$ to be about 5·4 per cent. of $P_{w\ \mathrm{plasmolysed}}$; a sufficiently close agreement for the hypothesis not to be rejected, although it is admitted that this is merely circumstantial evidence of limited extent.

II. Part of the interest attaching to measurements of water permeability of unplasmolysed cells is bound up with the question of existence of an 'active' component of turgor pressure. The view that turgor is increased in certain cases by some form of water-secretory activity of the living protoplast has been expressed by a number of authors (Bennet-Clark, Greenwood, and Barker, 1936; Bennet-Clark and Bexon, 1940, 1943; Brauner and Hasman, 1946, 1947, and others) and the nature of the evidence and of a considerable body of conflicting opinion is summarized clearly and fully by Crafts, Currier, and Stocking (1949) so that details need not be given here.

One of the more important points was raised by Levitt (1947), who determined the work which would have to be done to maintain an excess pressure on the hypothesis that continual pumping of water inwards was being carried out simultaneously with continual leakage of water outwards. This mechanism is the one which would consume most energy and is alternative to a mechanism consisting of a pump forcing water inwards and without any actual circulation due to leaks. Accepting the hypothesis of leakage which represents the greatest possible energy consumption, one can, as Levitt points out, show that the work done, W, in maintaining an excess active pressure, p, is given by

$$W = p.a.v.t,$$

where a is the area, v the velocity of water movement, and t is time.

Further

$$W_1 = p.A.P_w p,$$

where W_1 is work done in 1 hour by 1 g. of tissue and A is the specific area (area per g.) and P_w the water permeability, this last being an estimate of the rate of leakage.

For the beet tissue used in this work therefore

$W_1 = (5\times 10^6).(600).[(0\cdot 7\times 10^{-4}).5]$ where 'excess active pressure' is 5 atm. ($p = 5\times 10^6$ dynes, $A = 600$ cm.^{2}, $P_w p = 0\cdot 7\times 10^{-4}\times 5$).

$W_1 = 10\cdot 5\times 10^5$ ergs/g./hour.

W_1 similarly $= 0\cdot 42\times 10^5$ ergs/g./hour where the excess active pressure is 1 atm.

Levitt's values are based on unsatisfactory evaluations of A and P_w which he took as 240 cm.^{2} and 20×10^{-4} cm./atm./hour respectively. These give

$$W_1 = (5 \times 10^6).(240).[(20 \times 10^{-4}).5]$$

$$= 120 \times 10^5 \text{ ergs/g./hour, where the excess active pressure is 5 atm.}$$

A respiration rate of 50 μl./g./hour yields 102×10^5 ergs/g./hour, and in fact rates of respiration in beetroot disks vary from an initial value of around 35 μl. to 50 μl. rising fairly quickly to over 100 μl./g./hour.

Energy available in respiration is therefore considerably in excess of what would be needed to support 'active excess pressures' up to 5 atm. This fact alone in no way provides evidence of existence of an active pressure, but it eliminates the argument that active pressures are energetically impossible. Moreover, the energy requirement is calculated on the assumption of continued circulation of water in through certain channels and out through others which gives a maximum value.

ACKNOWLEDGEMENT

The writer wishes to express his gratitude to Professor Haldane for assistance in the statistical treatment of specific areas and to Professor Bennet-Clark for the suggestion of the problem and help in the course of the research.

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The Peltier Effect and its Use in the Measurement of Suction Pressure

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SUMMARY

1. A method is described by means of which the suction pressure of living material may be measured by observations on its equilibrium vapour pressure.

2. It employs a very fine thermocouple whose tip is cooled by means of the Peltier effect, below the dew-point. It collects a minute amount of dew, and can thus be used as an exceedingly fine 'wet-bulb' thermometer. As such it is convenient to use, quick in action, and causes very little disturbance to the system under observation.

3. The thermocouple is calibrated by means of salt solutions of known strength. The scale is fairly linear.

4. The method possesses the advantages of giving the suction pressure by direct observation rather than by interpolation; of speed in the actual measurement; of not subjecting the tissue to immersion; and of permitting organs like leaves, otherwise difficult to handle, to be easily dealt with. It also opens possibilities outside the range of the conventional methods.

INTRODUCTION

EVER since its introduction by Ursprung and Blum in 1916, the concept of suction pressure—or to give it two of its very numerous alternative names, suction force, or diffusion pressure deficit—has been the subject of much study and of a good deal of controversy. In another paper the writer has reviewed the suggestion that the name 'suction potential' should be used for this property, this term having apparently first been suggested by H. R. Oppenheimer (1930). The purpose of the present paper, however, is simply to describe a method for measuring this quantity which appears to have some advantages over the methods commonly used.

In general, measurements of suction potential have followed one or other of two main procedures. In the first, the suction potential has been balanced against an osmotic potential provided by solutions of sucrose or, more rarely and less satisfactorily, of salts. This procedure has involved the use of a series of solutions of graded strength, in which samples of the tissue or organ, as nearly alike as possible, were immersed. The solution in which there was no net gain or loss of water by the tissue was determined by interpolation from observations on some property of the solution (e.g. refractive index or density) or of the tissue (e.g. weight, length, or cell size). The osmotic potential or pressure of this solution was then equal to the suction pressure required. This general method has been the most widely used, but it suffers from the disadvantages that the tissue must be immersed (with unknown consequences), a fair amount of material is usually required, and the procedure is time-consuming and not very precise. Nevertheless, because of its simplicity and

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straightforwardness most suction-pressure determinations have followed this method.

The second general procedure requires only a single observation to be made. As a consequence the answer is obtained directly without interpolation and only one sample of the material is needed. It may be illustrated by the thermo-electric method of A. V. Hill (1930), later modified by E. J. Baldes (1934), for handling minute quantities of biological fluids. In A. V. Hill's method the quantity observed is actually a small temperature change (strictly, a galvanometer deflexion) produced by evaporation or condensation of moisture, and the instrument must be calibrated, but the suction potential could be observed directly as a pressure by a modification of another method due to R. V. Townend (1928).

The method now to be described follows the second general scheme, i.e. it enables the suction potential to be found by a single observation on one sample. Like the methods of A. V. Hill and R. V. Townend, it employs the fact that the lowering of aqueous vapour pressure over a solution or tissue can be used as a measure of the osmotic or suction potential, and like A. V. Hill's method the quantity actually measured is a minute change of temperature and the apparatus must be calibrated to yield an answer in atmospheres. It possesses a number of advantages, the chief of which being that no multiplication of samples is required, the subject is not immersed in solutions but kept in moist air, and equilibrium is attained rapidly. Further, the arrangement permits the gaseous atmosphere to be varied at will, and thus the influence of oxygen starvation, anaesthesia, &c., can be investigated. Lastly, it simplifies the handling of such organs as leaves, the suction potentials of which it permits to be measured very rapidly and easily.

THE PELTIER EFFECT

Before describing the method it may be as well to recall a few facts of elementary physics. When two metals are placed in contact they assume, in general, a difference of electrical potential, the so-called Peltier coefficient. Peltier discovered in 1834 that on passing a current across such a junction there is a liberation or absorption of heat at the point of union—the temperature rising or falling according as the current flowed down or up the potential gradient. If P is the Peltier coefficient (in volts) and i the current (in amperes), the rate of liberation or absorption of energy at the junction is Pi watts. When the current flows there are naturally two factors to consider: firstly, the irreversible heating of the conductors by the ordinary Joule effect (this being proportional to the square of the current), and secondly, the reversible heating or cooling by the Peltier effect (proportional, as mentioned above, to the first power). From this it follows that the degree of *cooling* which can be obtained by using the Peltier effect is limited—it will increase with the current only up to the point at which the Joule heating begins to predominate.

Suppose now that the junction of a fine thermocouple is placed in an atmosphere of very humid air, and a current passed in the sense required to cool it.

Then, if the degree of cooling is sufficient to bring the junction below the dew-point, moisture will condense on it and the thermocouple will become potentially a delicate 'wet-bulb' thermometer. The dew will re-evaporate on breaking the circuit and a minute e.m.f. will be generated more or less proportional to the 'wet-bulb' depression associated with the atmosphere surrounding the junction. If a sensitive galvanometer be connected to the thermocouple, it would seem possible to calibrate this apparatus by enclosing the junction in a chamber containing solutions of known osmotic strengths, and once calibrated, it could be used for measuring the suction potential of living tissues. This suggestion was tried and eventually proved to be practicable, but before describing the method evolved and some results obtained with it, one or two points of theoretical interest will be discussed; where these involve mathematical derivation, this will be relegated to Appendix I.

Degree of cooling attainable

As mentioned earlier, the degree of cooling which the Peltier effect is capable of producing is limited by the incidence of the ordinary Joule heating effect. It is, in fact, a quadratic function of the current, and shows a maximum which depends principally on the square of the thermo-electric power (see Appendix I). A few values for typical thermocouples follow:

Iron–Constantan	1·6° C.
Antimony–Bismuth	6·9° C.
Manganin–Constantan	0·6° C.
Bismuth–Bismuth + 5% tin	4·9° C.

Provided the wires are long and suitably proportioned, the above values are independent of the size of the couples: they may, however, be as much as doubled if the wires of the couple are short (see Appendix I).

Relation between osmotic pressure and dew-point depression

It can be seen from the above that the Peltier effect is capable of affording only a small degree of cooling, and it is therefore of interest to see just how far the dew-point is depressed over solutions whose osmotic pressure lies in the range of interest to the physiologist. Taking a solution whose osmotic pressure is 10 atmospheres, approximate calculation from the usual thermodynamic formulae gives the depression of the dew-point as about 0·124° C. at 25° C., the corresponding relative humidity being about 99·24 per cent. For a dew-point depression of 1° C., therefore, the osmotic pressure would be about 80 atm., from which it appears that, theoretically at least, dew could easily be condensed from above even quite strong physiological solutions. On the other hand, it is clear that if suction potentials are to be measured to, say, 0·1 atm., the apparatus must be capable of distinguishing dew-points or wet-bulb temperatures to a very fine degree of accuracy, i.e. to the order of 0·001° C. or so.

Galvanometer sensitivity and thermostatic control

This fact will govern the sensitivity necessary in the galvanometer circuit. Supposing that a thermocouple be employed having a thermo-electric power of 100 μV per 1° C., then the circuit must be capable of detecting fairly easily 0·1 μV in order to be capable of measuring suction potential differences of 0·1 atm. Allowing 1 mm. on the galvanometer scale for this increment, and assuming that the resistance of the couple is appreciably less than that of the galvanometer, the sensitivity required for the latter works out at about 1 mm. per 0·1 μV, or 10 mm. per μV. This is fairly easily attained in practice with a good instrument, but the speed of response must not be reduced too much: the galvanometer period should preferably not be much above 2–3 seconds.

Besides sensitivity in the galvanometer circuit it is obvious that the instrument must be operated in a temperature-bath controlled to rather finer limits than usual. The necessity for this will be more apparent from what follows.

Suppose the thermocouple to be enclosed in a small chamber with a sample of tissue, the whole being immersed in a water-bath at temperature T.

Let the equilibrium vapour pressure above the sample of tissue be p; then for the purpose of this inquiry this can be taken as the vapour pressure throughout the chamber.

Further, let the saturation deficit at the thermocouple (i.e. the difference between p and the maximum vapour pressure p_0 of water at T) be σ.

Then the galvanometer deflexion θ can be considered as a function of the temperature T and the saturation deficit σ in the vicinity of the junction. We can therefore write:

$$d\theta = \left(\frac{\partial\theta}{\partial\sigma}\right)_T d\sigma + \left(\frac{\partial\theta}{\partial T}\right)_\sigma dT.$$

Now the 'wet bulb' depression is known to vary little with the temperature, at constant saturation deficit; hence it would seem reasonable to ignore the second term above. This gives:

$$d\theta = \left(\frac{\partial\theta}{\partial\sigma}\right)_T d\sigma,$$

and this can be expanded into

$$d\theta = \left(\frac{\partial\theta}{\partial p}\right)_T \left(\frac{\partial p}{\partial\sigma}\right)_T d\sigma,$$

or remembering that $\sigma = p_0 - p$,

$$d\theta = \left(\frac{\partial\theta}{\partial p}\right)_T (-1)\, dp_0$$

$$= -\left(\frac{\partial\theta}{\partial p}\right)_T \left(\frac{dp_0}{dT}\right) dT,$$

where in evaluating $d\sigma$ the temperature of the tissue (and so p) is assumed to remain unchanged, while the thermocouple rises by the increment dT.

Suppose the instrument gives an increment of deflexion of 1 mm. for an increment of 0·1 atm. in the osmotic pressure at 25° C. Then $(\partial\theta/\partial p)_T$ for ordinary values of the osmotic pressure can be calculated to be about 1 mm. change in deflexion per 0·00175 mm. Hg variation in p. Again, at 25° C. dp_0/dT for water is about 1·41 mm. Hg/° C.

Hence for a variation dT of 0·001° C. between the temperatures of the tissue and the thermocouple,

$$d\theta = \frac{1{\cdot}41}{0{\cdot}00175} \times 0{\cdot}001$$

$$= 0{\cdot}8 \text{ mm.}$$

This error in the galvanometer reading is appreciable: it shows that the thermostat bath, for accurate work, should be controlled to 0·001° C. at least.

Behaviour of a thermocouple in moist air

When the feasibility of the present method had been established from a purely theoretical point of view, it remained to be ascertained whether it would work in practice. The first step in this direction was to make a suitable thermocouple with a much finer couple attached to its free end and serving to indicate its temperature changes (Fig. 1).

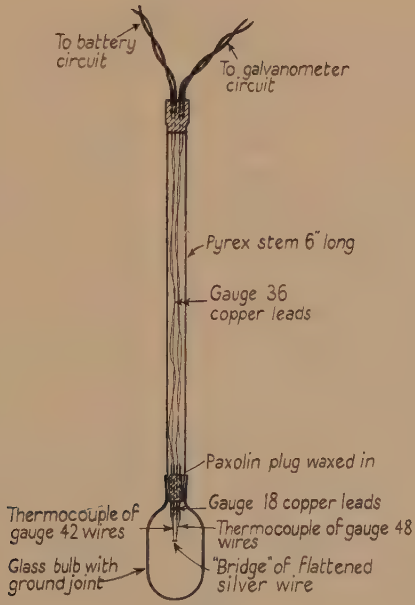

FIG. 1.

A small volume of a salt solution was placed in the bulb and the latter immersed in a thermostat. With this simple set-up, and the circuit shown in

Fig. 2, the reactions of the thermocouple tip on passing a current which cooled it in the presence of humid air were investigated. They are represented graphically in Fig. 3, and may probably be taken as indicating the general course of behaviour of any junction subjected to cooling in air of sufficiently high humidity.

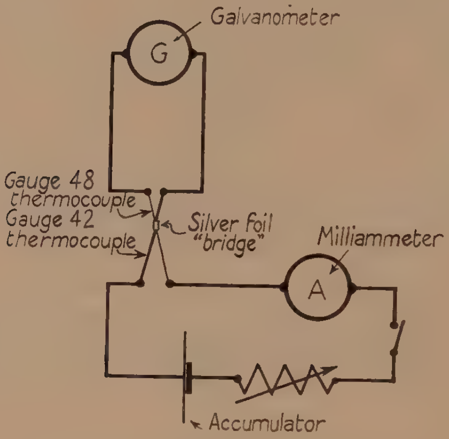

FIG. 2.

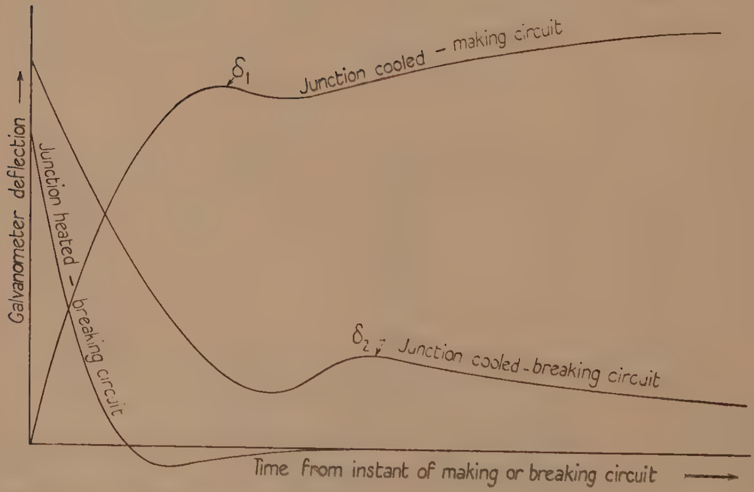

FIG. 3. Note: Deflexions when junction is heated are in opposite directions.

It will be noticed that on breaking circuit the temperature of the tip rises at first rapidly and then very slowly towards the ambient temperature, whereas when the current has been reversed and the junction heated the ambient temperature is reached very quickly. This seemed definitely to indicate dew formation, a conclusion which was confirmed in many other ways. It will be noticed, too, that on all the curves there is a definite 'hump'. This was investigated mathematically and the cause traced to the interaction between the Peltier and Joule effects (see Appendix I). It was found that the galvanometer readings corresponding to these 'humps' (i.e. to δ_1, and δ_2, Fig. 3) were linear functions of the molar strength of the salt solution, and these deflexions could therefore, if desired, be used to measure osmotic or suction pressures. The ideal circuit for this would probably be a simple Wheatstone bridge, but as a suitable apparatus was not available, this suggestion was not pursued.

'Ballistic' method of operation

Instead of observing the temperature of the junction tip continuously another possible method of operation suggested itself. It was tried and finally adopted; for convenience it can be called the 'ballistic' method. The very simple circuit for this is shown in Fig. 4, and the method of operation was as follows. At a given instant a rapid-action switch was closed and a stopwatch started. A predetermined current was allowed to flow through the thermocouple for a definite time (say 5 or 10 seconds) and then the switch was released, breaking the current and instantly putting the couple into circuit with a galvanometer, whose 'ballistic' throw was recorded. The galvanometer returned only slowly to zero; when it had done so, however, a repeat reading was made.

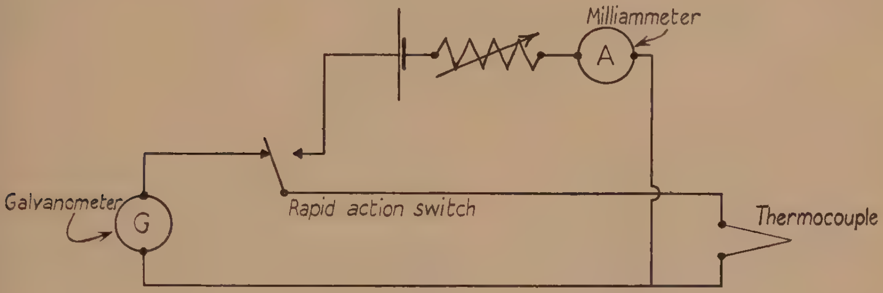

FIG. 4. Circuit for the 'ballistic' method.

In trying out this method it was first necessary to ascertain the general behaviour of the instrument when used in this way, and then, if satisfactory, to fix suitable values for the strength and duration of the current. This required a fairly detailed investigation of the properties of the thermocouple to be used, and the results for an assembly similar to the one shown in Fig. 11 (below) are given graphically in Figs. 5 to 8.

To investigate the effect of varying the current strength, about 1 or 2 c.c. of a salt solution was introduced into the chamber, the instrument placed in the water-bath, and allowed to come to temperature equilibrium. The current was then adjusted to have various values from zero to 5 mA; each time it was allowed to flow for a fixed interval, and was then interrupted and the 'ballistic' throw observed. The results are plotted in Fig. 5, the solution in the chamber being 0·5 M $BaCl_2$, and the current duration 10 sec.

It will be observed from these curves that the galvanometer responded very differently when the junction was heated rather than cooled—another indication that in the latter case dew was actually being condensed. At quite a low value of the current the 'cooled' curve begins to rise quite suddenly, very soon, however, flattening out and forming a 'knee'. With solutions of lower strength the 'knee' is not always so pronounced; it moves progressively to the right as the molar strength increases (see Fig. 6). As the current is still further increased the deflexion may or may not go on rising, and it may show reversals on breaking circuit. These reactions are probably due to several causes; the junctions of the copper leads with the thermo-electric wires begin to change in temperature at high currents, and the free tip itself reverses its cooling (see Appendix I). However, in practice this stage is never reached: the current is most conveniently kept low.

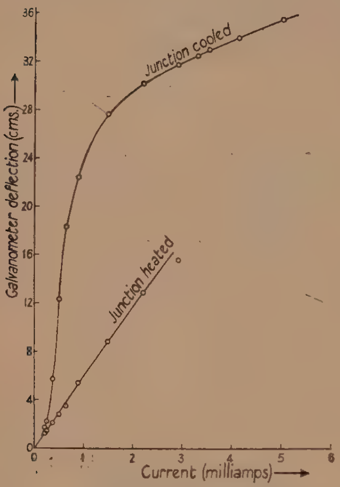

FIG. 5. Effect of current on galvanometer deflexion. Solution, 0·5 M $BaCl_2$; duration of current, 10 sec.; temperature, 26·1° C. Note: deflexions for two curves are in opposite directions.

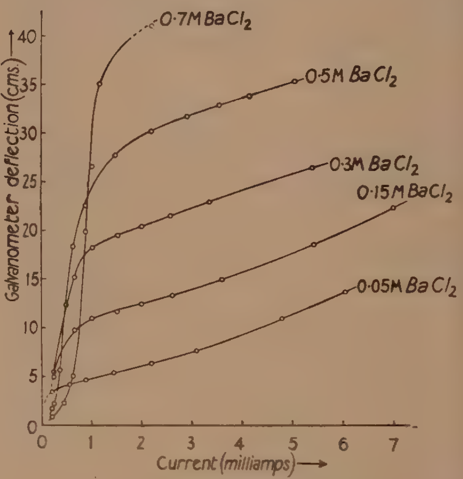

FIG. 6. Effect on deflexion curves of varying humidity. Duration of current, 10 sec.; temperature, 26·1° C; solutions as marked.

The effect next of varying the duration of the current was investigated. Fig. 7 shows typical results for a different thermocouple.

It will be observed that the same phenomenon of a steep rise followed by a flattening out occurs both when the current is increased and when its duration is lengthened. It may be surmised that the reason for this is as follows. The galvanometer takes a finite time to swing out. When only a small quantity of dew has been condensed all this will have re-evaporated before the galvanometer has reached its full swing. In this stage the deflexion is very sensitive to the quantity of dew collected. When, however, the current or the duration have been increased so much that not all the dew has gone by the time the galvanometer is at its farthest swing, the deflexion becomes much less sensitive to changes in the moisture condensed and the curves flatten out. The practical point, of course, is that the values of current and time, while being naturally as low as possible to avoid observational disturbances to the system being investigated, should nevertheless be sufficiently far to the right of the 'knee' to ensure that errors in either will not greatly affect the result.

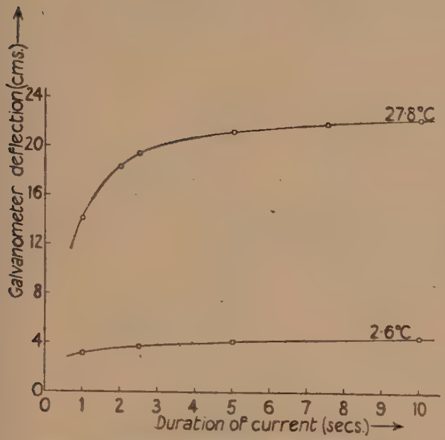

FIG. 7. Effect of varying the duration of the current. Solution, 0·6 M KCl; current, 2·00 mA; temperatures as shown.

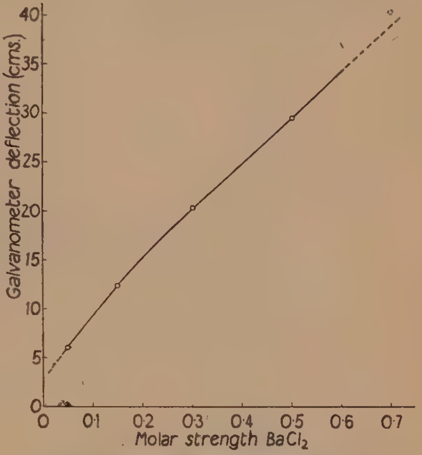

FIG. 8. Variation of galvanometer deflexion with osmotic strength. Current, 2·00 mA; duration, 10 secs; temperature, 26·1° C.

Having chosen suitable values for the current and the time, the instrument was then tried using solutions of different strengths. A typical result is shown in Fig. 8, which was got with a couple earlier than the one shown in Fig. 11. The current was 2 mA and the time 10 sec.

The result—one of many similar ones—clearly showed that the method has not only theoretical but also practical possibilities and justified further work on it. This has taken the method to the stage in which it can be of definite value, and the set-up now in use will be described.

DESCRIPTION OF APPARATUS

Temperature bath

The apparatus may conveniently be divided into three parts: the thermostat bath, the galvanometer and the thermocouple circuit, and the thermocouple assembly. These will be dealt with in order.

As mentioned above, a thermostat bath controlled to finer limits (0·001° C.) than usual is required. The essentials for such a bath may be listed as:

1. Very good lagging and prevention of evaporation.
2. Rapid stirring.

3. Temperature-detector very sensitive and both it and heater with quick response times.
4. Adequate volume of water or other fluid.
5. Preferably proportionate control of heat input.

These requirements were met by using a rectangular glass tank holding about 20 litres of water. This was placed inside a stout wooden box and the space between the two (about 1½ in. to 2½ in. wide) was packed with kapok,

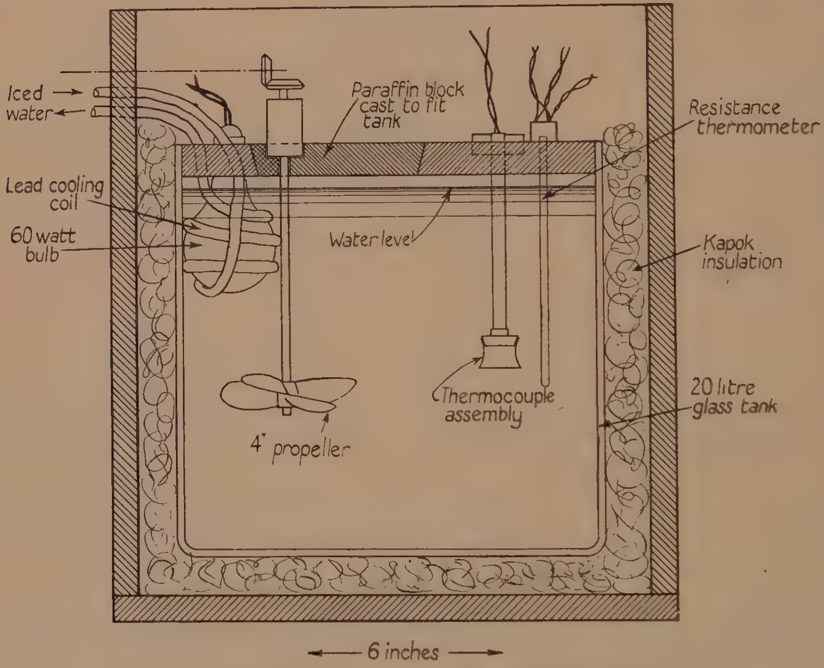

Fig. 9. Arrangement of temperature bath. Approx. to scale.

the outer walls of the tank and the inner ones of the box having first been covered with tinfoil. To hinder loss of heat by evaporation from the water surface a slab of paraffin wax about 1 in. thick was cast to fit tightly inside the tank at the top: through this were drilled holes for the thermometer, stirrer shaft, &c. The bath was stirred by a 4-in. diameter propeller. A 60-watt lamp was used as heater, this having the advantages of low heat capacity and of giving off its energy by radiation, this latter ensuring a rapid and general rather than local heating.

The temperature-detecting element was a small resistance thermometer wound with two 10-ohm coils of 44-gauge enamelled copper wire and two 10-ohm coils of 34-gauge manganin. The whole was encased in a thin copper sheath and formed a bridge balancing at about 27·8° C., the temperature of balance being adjustable by shunting one of the copper or manganin arms. The thermometer was put in series with 50 ohms and supplied with 2 volts, when it produced a deflexion on an old Paul mirror galvanometer of about 2·4 mm. per 0·001° C.

The galvanometer was arranged to reflect its spot of light on to a gas-filled photocell incorporated in a phase-control thyratron circuit (A. W. Hull, 1929; see also C. E. Waring and G. Robinson, 1943). This arrangement was very stable and worked admirably; it was easy to get a smooth change in the heater input from zero to 30 watts with a total change of bath temperature of less than 0·005° C.

When the bath was running the movement of the spot of light was rarely more than 1 mm., corresponding to about 0·0004° C. The general arrangement of the bath is shown in Fig. 9.

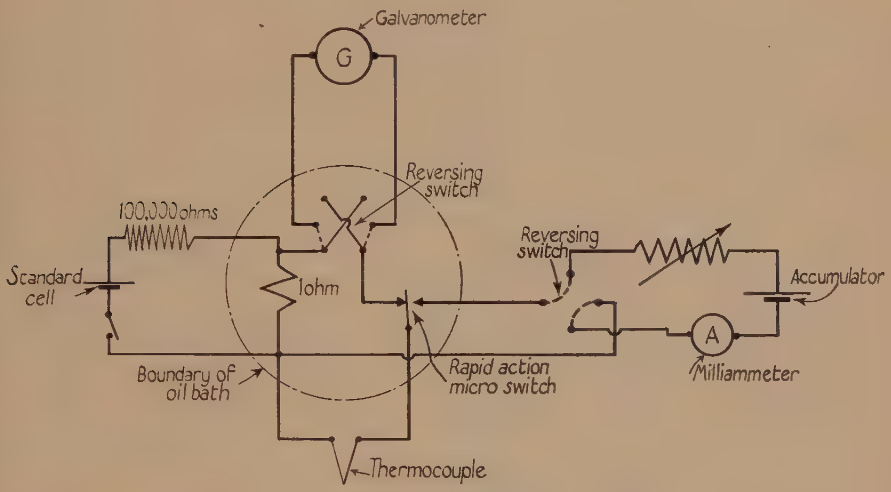

FIG. 10. Circuit for the 'ballistic' method.

The galvanometer and thermocouple circuit

The actual circuit used with the thermocouple is characterized by great simplicity (Fig. 10).

The thermocouple is arranged to be normally in series with a sensitive galvanometer G, the instrument used being a Kipp Zernicke of the following characteristics:

Resistance . .	14·5 ohms.
Period , .	2·5 seconds.
Sensitivity . .	about 10–15 mm. per μV at 1 metre as used. (It was always employed with the magnetic shunt set for critical damping.)

When the microswitch is depressed the couple is thrown out of circuit with the galvanometer and connected to an accumulator or large dry cell, a milliammeter and a plug-type resistance box being also included in the circuit to enable the current to be fixed at any desired value. On releasing the microswitch the current is broken and the thermocouple instantly connected to the galvanometer. There is also a circuit incorporating a standard cell and high resistance to enable the sensitivity of the galvanometer to be checked at intervals, and a commutator to reverse the galvanometer connexions when testing. The portion of the circuit enclosed within the dotted line was immersed in an oil bath to reduce parasitic thermal e.m.f.'s, which, however, were not troublesome. Much more trouble was experienced with leakage currents from the D.C. mains, and even when the whole apparatus including the water-bath was stood on aluminium sheeting, rapid movements of the galvanometer were sometimes apparent.

The thermocouple assembly

In designing the thermocouple assembly several points had to be kept in mind. With regard to the thermocouple itself it was obviously desirable to make it as small as convenient, since not only does any air movement then have much less effect on the 'wet-bulb' reading (R. W. Powell, 1936), but speed of response is increased and the disturbing effect of the junction on the surrounding moist atmosphere is reduced. Again, the wires should be short and connected to relatively massive copper leads, since this not only increases the available degree of cooling of the free junction (by conducting away the heat which inevitably accompanies it elsewhere in the circuit—see Appendix I), but it also reduces the electrical resistance and so the waste heat liberated on the passage of the current. Further, it virtually limits change of temperature to the *free* junction. The most satisfactory materials for the wires appeared to be bismuth and an alloy of bismuth with 5 per cent. tin. This combination has a high thermo-electric power; the wires can easily be drawn out fine by the Taylor process (see Strong, 1938); they can be readily soldered with Wood's metal or welded by a condenser discharge, and they are stable in moist air.

Fig. 11 shows details of construction of a typical apparatus. Only one thermocouple was in fact used at a time, but there is no reason why the two should not have been connected in series or, as mentioned above, in a Wheatstone net. Several other couples were made: one with extremely fine wires of about 5 μ diameter had a resistance of 70 ohms, but it was not generally so satisfactory as the one shown.

So far as the thermocouple chamber and mounting is concerned, apart from designing it to be airtight and capable of standing immersion in the water-bath, the principal requirements were to ensure that all parts of it, including the fine thermocouple leads, should rapidly attain to the bath temperature, and that the chamber should be as small as practicable to reduce the time taken to establish vapour-pressure equilibrium within it.

It was desirable that the flexible leads should be connected in such a way as to avoid junctions likely to produce parasitic thermal e.m.f.'s: this was done by arranging them to be enclosed within the heavy brass tube carrying the chamber.

In the assembly shown, each couple has a resistance of about 6 or 7 ohms between the flexible leads, and the bismuth and alloy wires have diameters of about 25 μ and 35 μ respectively and lengths of about 1 mm.

The volume of the thermocouple chamber is roughly 13 c.c.: it requires, therefore, about 0·3 mg. of water vapour at 25° C. to fill it.

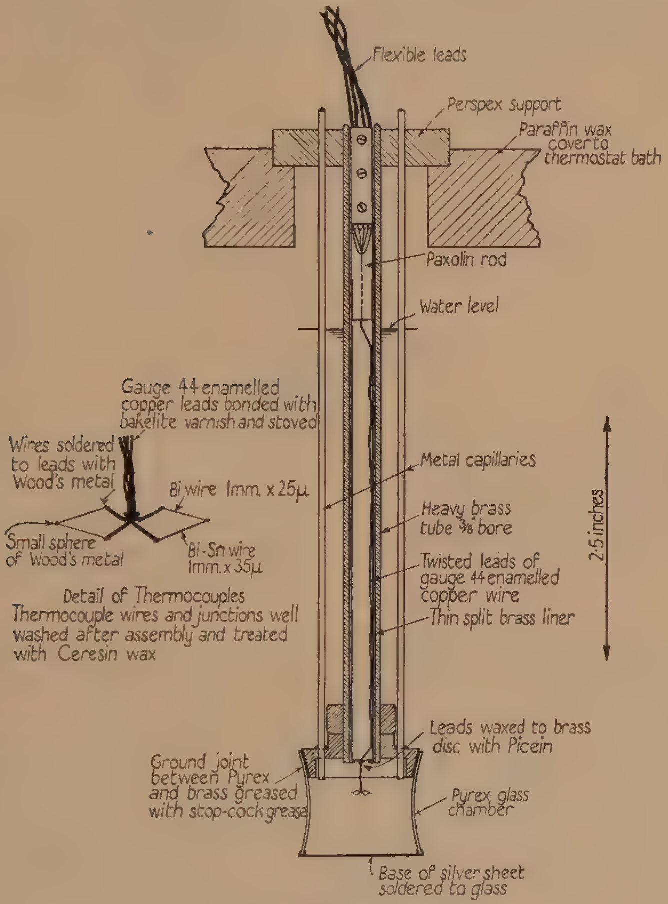
Flexible leads
Perspex support
Paraffin wax cover to thermostat bath
Paxolin rod
Water level
Gauge 44 enamelled copper leads bonded with bakelite varnish and stoved
Wires soldered to leads with Wood's metal
Bi wire 1mm. x 25μ
Small sphere of Wood's metal
Bi-Sn wire 1mm. x 35μ
Detail of Thermocouples
Thermocouple wires and junctions well washed after assembly and treated with Ceresin wax
Metal capillaries
Heavy brass tube ⅜" bore
Twisted leads of gauge 44 enamelled copper wire
Thin split brass liner
2·5 inches
Leads waxed to brass disc with Picein
Ground joint between Pyrex and brass greased with stop-cock grease
Pyrex glass chamber
Base of silver sheet soldered to glass

FIG. 11. Thermocouple assembly.

SUCTION POTENTIAL MEASUREMENTS OBTAINED WITH THE APPARATUS

This part of the paper may conveniently close with a description of one or two experiments carried out with the help of the present method.

Fig. 13 shows an early experiment performed on a leaf of *Forsythia* (15 Sept. 1947). The leaf was rolled into a cylinder supported with axis vertical in a little wire frame in which it could be rapidly weighed. It was weighed on removal from a moist covered Petri dish in which it had spent some hours and was then at once dropped into the thermocouple chamber and placed in the water-bath. The thermocouple protruded into the atmosphere within the leafy cylinder. As soon as thermal equilibrium was established (about 10 or 15 min. as judged by the absence of any deflexion on the galvanometer connected to the couple) 'ballistic' readings were taken using a current of 2·45 mA flowing for 10 sec. These were continued at intervals till they became constant. The chamber was then opened, the leaf weighed, subjected to drying in a desiccator, and then again returned to the chamber for measurements of its suction pressure. This procedure was repeated six times, and the results are shown plotted, the times against the various curves representing total durations of desiccation, and the galvanometer deflexions having been converted to atmospheres of equilibrium suction pressure by means of the calibration curve shown in Fig. 12. It will be observed firstly that the mean suction potential of the leaf increases with drying, as would be expected; and also that whereas vapour-pressure equilibrium in the chamber is reached very rapidly with the leaf turgid, it becomes a slower and slower process as the leaf dries. Why this is so is a matter of conjecture, but it may be supposed that both stomatal closure and the physical state of the cell walls have a part in it. It is instructive to plot the values of the suction potential (read from the flat part of these curves) against the loss in water of the leaf. When this is done the result shown in Fig. 14 is obtained. A distinct 'knee' is observable in this curve, presumably marking the region of water content in which flaccidity begins.

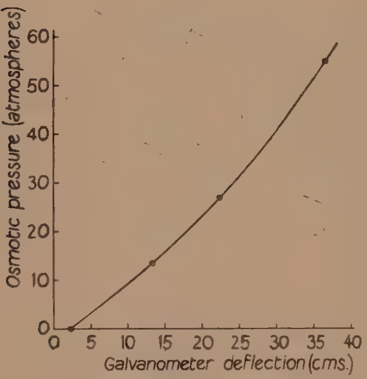

FIG. 12. Calibration curve. Temperature 26·1° C.

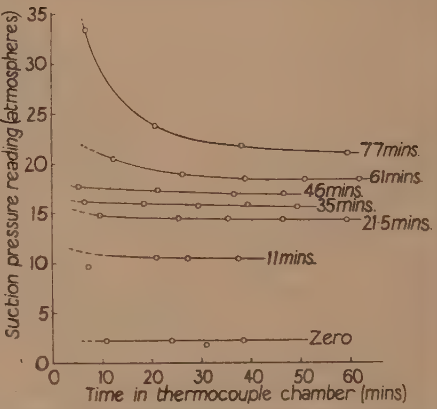

FIG. 13. Behaviour of a leaf of *Forsythia*, on wilting. Temperature 26·1° C. Times shown on curves are total times of drying.

A second experiment of interest was designed to test the hypothesis of Bennet-Clark of an 'active' component affecting the water-absorbing power of plant cells, though it subsequently appeared that it was not well suited to do this. The bath was arranged to run at temperatures below ambient as well as above by placing a small coil of lead tubing around the heater, and running a stream of iced water through it under constant head. The temperature control was by the heater as before. Solutions of potassium chloride of graded strength were then placed successively in the instrument, and galvanometer readings taken at various temperatures between 2° C. and 35° C. This enabled curves to be drawn showing how the instrument reading for solutions of constant strength varied with temperature. Having determined a series of such lines, living tissues were substituted for the chloride solutions, with the secret hope that a different sort of behaviour would be observed in their reactions to temperature. However, no such difference manifested itself; Fig. 15 shows the results for a young wheat leaf and for a thin slice of potato, together with curves for 0·2, 0·4, and 0·6 M (w/w) KCl. It is obvious that these curves all belong to more or less the same 'family', and indeed it would seem that even if the cells possessed an active water-absorbing mechanism in addition to the ordinary osmotic one, its effect on the equilibrium vapour pressure would at least be problematical.

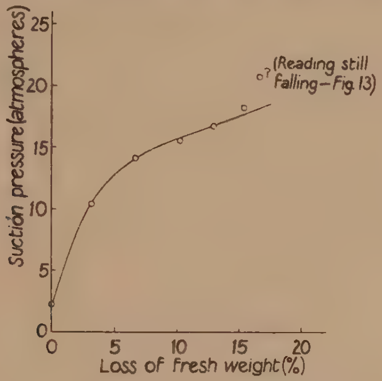

FIG. 14. Variation of suction pressure of *Forsythia* leaf on wilting.

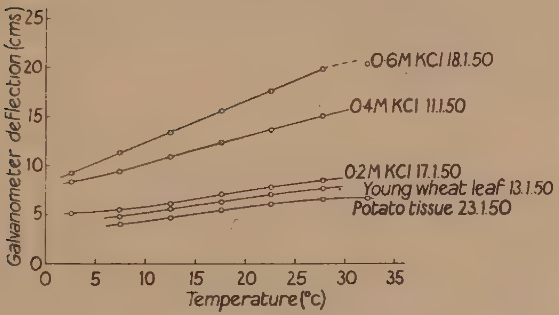

FIG. 15. Effect of temperature on galvanometer deflexion.

CONCLUSION

In conclusion it may be said that the present method for measuring suction potentials, while it requires a more elaborate set-up than the methods hitherto used, does seem to possess advantages of its own and to be capable of lending itself to the investigation of lines of approach otherwise almost out of reach. The problem of water movement is potentially so complex and many-sided that any new experimental technique is worth considering in an effort to solve it.

So far as practical matters are concerned one or two points might be mentioned. The technique sometimes seems to yield erratic results—repeat observations differ more widely than they ought. There are a number of causes for this, the principal being (i) repeat readings taken too soon, i.e. before the moisture-laden tip has really returned to equilibrium with the atmosphere; (ii) variations in current and timing, especially if the steep parts of the curves of Figs. 5, 6, and 7 are operated on; (iii) lack of temperature equilibrium with the bath; (iv) lack of vapour-pressure equilibrium inside the chamber (see e.g. Fig. 13); (v) not sufficiently good thermostatic control; (vi) presence of pure condensed water on the sides or top of the chamber—almost certain to occur if the chamber is left with materials in it and not under close thermostatic control.

The galvanometer deflexion does not follow any simple law. Under some conditions it agrees very well with the calculated 'wet-bulb' depression, but obviously the 'thermal inertia' of the tip is a factor as well as the wet-bulb lowering, and it will be of increasing importance as the latter is reduced. It is obviously the principal, almost the only factor influencing the reading obtained over pure water (Fig. 8).

APPENDIX I

Mathematical Theory of Cooling by the Peltier Effect

Referring to Fig. 16, let OX_1, OX_2 be the two elements of a thermocouple, joined at O, and let us assume that they are uniform circular wires of semi-infinite extent. Consider the wire OX_1:

Let x = distance along the wire from the junction to M,
θ = temperature depression of the wire at M below the ambient temperature,
P = Peltier coefficient between the metals of the wires,
K = thermal conductivity of the material of the wire,
ρ = specific electrical resistance of the same,
A = cross-sectional area of the wire,
a = its perimeter,
E = emissivity of its surface, i.e. rate of heat loss or gain per unit area per unit temperature difference between the wire and its surroundings,
i = current flowing in the wires.

Of these quantities, x will normally be in cm., θ in °C., P in volts, K in watts per cm. per °C., ρ in ohm–cm., A in cm.^{2}, a in cm., i in amperes, and E in watts per cm.^{2} per °C.

The Thomson effect is neglected in the following treatment as being insignificant in the present connexion.

Consider the element of wire MN. When the current has been passing a sufficient length of time to establish a steady state, the heat input to this element by the Joule effect, by conduction across N and by communication from the surroundings must exactly balance that lost by conduction across M. In symbols

$$i^2\frac{\rho\delta x}{A}+KA\left(-\frac{d\theta}{dx}\right)_N+E\,a\,\delta x\,\theta = KA\left(-\frac{d\theta}{dx}\right)_M, \tag{1}$$

where $(-d\theta/dx)_{M,N}$ are the temperature gradients at M and N respectively.

Rearranging the above equation gives

$$KA\left\{\frac{(d\theta/dx)_N-(d\theta/dx)_M}{\delta x}\right\}-Ea\theta-\frac{i^2\rho}{A}=0,$$

or in the limit when $\delta x \to 0$

$$KA\frac{d^2\theta}{dx^2}-Ea\theta-\frac{i^2\rho}{A}=0. \tag{2}$$

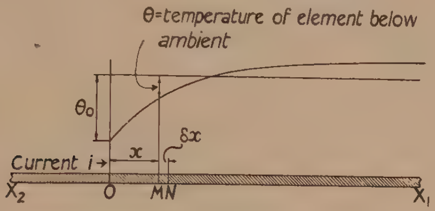

FIG. 16.

The general solution of this equation may be written in the form:

$$\theta = C_1e^{-\alpha x}+C_2e^{\alpha x}-\frac{i^2\rho}{EaA}, \tag{3}$$

where $\alpha = \sqrt{(Ea/KA)}$ and C_1 and C_2 are constants.

For our particular case the boundary conditions determining C_1 and C_2 are:

(i) when $x \to \infty$, $d\theta/dx$ must tend to zero;

(ii) at the junction ($x = 0$) heat is absorbed at the rate Pi, and this is supplied by conduction, partly along the wire X_1O and partly along X_2O. The relative proportions passing along the two wires will depend on their properties: suppose the fraction λ to pass from the wire X_1O.

The rate of conduction along the wire towards O at any point is

$$KA\left(-\frac{d\theta}{dx}\right),$$

and consequently the second condition leads to

$$KA\left(-\frac{d\theta}{dx}\right)_{x=0}=\lambda Pi.$$

Condition (i) gives $C_2 = 0$; condition (ii)

$$KA\,C_1\alpha = \lambda Pi$$

or

$$C_1=\frac{\lambda Pi}{\sqrt{(KAEa)}}.$$

Hence equation (3) becomes

$$\theta=\frac{\lambda Pi}{\sqrt{(KAEa)}}e^{-\alpha x}-\frac{i^2\rho}{EaA}. \tag{4}$$

The value of λ may be found as follows. For $x = 0$, θ can be expressed either in terms of λ_1, K_1, A_1, E_1, &c., or in terms of λ_2, K_2, A_2, E_2, &c., where the suffixes stand for the two wires. Equating these results, and remembering that $\lambda_1+\lambda_2 = 1$, we can find λ_1 and λ_2 as two rather cumbrous expressions, linear functions of the current. However, it can be shown that there is an optimum proportion for the two wires, and with this, λ_1 (and of course λ_2) becomes independent of the current, and of value

$$\lambda_1 = \frac{\sqrt{(K_1\,\rho_1)}}{\sqrt{(K_1\,\rho_1)}+\sqrt{(K_2\,\rho_2)}}, \tag{5}$$

an analogous expression holding for λ_2.

The optimum proportion, for circular wires of radii r_1, r_2, turns out to be

$$\frac{r_1}{r_2} = \sqrt[3]{\frac{\rho_1}{\rho_2}}. \tag{6}$$

Since we are only interested in the temperature of the junction ($\theta = \theta_0$), we can write $x = 0$ in equation (4). This shows that θ_0 is a parabolic function of i exhibiting a maximum value (θ_{max}) occasioned by the negative term in i^2 (the Joule effect).

By the usual method the current giving the greatest degree of cooling is found (assuming (6) to hold) to be

$$i_{\text{max}} = \frac{\pi P}{\sqrt{(K_1\,\rho_1)}+\sqrt{(K_2\,\rho_2)}}\sqrt{\left(\frac{Er^3}{2\rho}\right)}, \tag{7}$$

and the maximum degree of cooling

$$\theta_{\text{max}} = \frac{P^2}{4\{\sqrt{(K_1\,\rho_1)}+\sqrt{(K_2\,\rho_2)}\}^2}. \tag{8}$$

Since by the Wiedemann–Franz law the product $K\rho$ for most metals and alloys is roughly the same, we can simplify (7) and (8) to a fairly close approximation by writing $\overline{K\rho}$ for the mean value of this product for the two metals. This leads, with a further small approximation, to

$$\theta_{\text{max}} = \frac{P^2}{16\overline{K\rho}}, \tag{9}$$

$$i_{\text{max}} = \frac{\pi P}{\sqrt{(\overline{K\rho})}}\sqrt{\left(\frac{Er^3}{8\rho}\right)}, \tag{10}$$

$$\lambda_1 = \frac{1}{2}\sqrt{\left(\frac{K_1\,\rho_1}{\overline{K\rho}}\right)}. \tag{11}$$

These results are applicable to an ideal couple of long wires whose diameters are in the optimum ratio (equation (6)).

Couples other than ideal

Two departures from the ideal couple can be considered.

(i) the junction itself not ideal, but presenting a finite area to the surroundings (e.g. a sphere of metal may embrace the ends of the two wires);

(ii) the wires may be of finite length.

With regard to (i), assume that attached to the junction O is a 'dead' area of perfectly conducting metal. This will be everywhere at the temperature of the junction: and its effect will be, by absorbing heat from the surroundings, effectively to lower the degree of cooling. It will absorb heat at the rate

$$Q = Eal\theta_0,$$

where al is its surface area.

This must be subtracted from the quantity Pi in equation (4). When this is done and the resulting equation solved for θ_0 it appears that the effect of the 'dead area' of the junction is simply to reduce the depression of the tip in the ratio $1 : (1+\lambda_1\,\alpha_1\,l_1)$, where λ_1 and α_1 have the usual meanings and l_1 is the length of OX_1 equal in surface area to the 'dead area' ($a_1\,l_1$) considered.

With regard to wires of finite lengths the general solution (3) will still apply, but the boundary conditions will be different. If the ends X_1, X_2 be considered as joined to massive copper leads, the boundary conditions may be written:

(i) $\theta = 0$ for $x = L$, very nearly

(ii) as before.

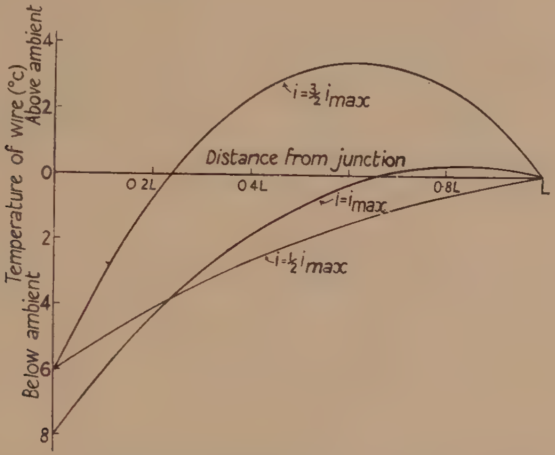

FIG. 17. Variation of temperature along wires. For details of thermocouple see text. $L = 2$ mm. approx.

The equation for θ becomes especially simple if the lengths L_1, L_2 of the wires are such that

$$\alpha_1 L_1 = \alpha_2 L_2$$

where, as before, $\alpha = \sqrt{(Ea/KA)}$. The solution then is

$$\theta = \frac{\lambda P i}{\sqrt{(KAEa)}} \frac{\sinh \alpha(L-x)}{\cosh \alpha L} - \frac{i\rho^2}{EaA}\left(1 - \frac{\cosh \alpha x}{\cosh \alpha L}\right), \tag{12}$$

where λ, again, is given by (5). Putting $x = 0$ in this the optimum current turns out to be

$$i_{\max} = \frac{\pi P}{\sqrt{(K\rho)}} \sqrt{\left(\frac{Er^3}{8\rho}\right)} \coth \frac{\alpha L}{2}, \tag{13}$$

and the maximum cooling obtainable

$$\theta_{\max} = \frac{P^2}{16K\rho}(1 + \operatorname{sech} \alpha L). \tag{14}$$

From this it appears that as the wires are reduced in length the optimum current increases continuously, and the degree of cooling rises to a limit of twice its previous value ($\operatorname{sech} \alpha L \to 1$ as $L \to 0$).

These results will be clearer if the variation of the temperature along the wire is calculated for a concrete example and the results summarized in graphical form. Assuming, therefore, wires of bismuth and bismuth–tin alloy, of diameters 25μ and 34μ respectively, and assuming that the length of each wire is adjusted to make the products αL each unity (this will make L_1 and L_2 each about 2 mm.), the variation of temperature along the wires has been plotted in Fig. 17 for three values of the current. Owing to the finite lengths of the wires the optimum cooling has been increased from 4·9° C. (Appendix 2) to 8·1° C., and the optimum current from 1·5 mA to 3·2 mA. The results have been calculated from equations (12), (13), and (14).

Time relation of the temperature changes

At one point in the work it became necessary to investigate how rapidly the thermocouple responded when a current was either started or stopped; this has a bearing on the 'humps' observed in the deflexion curves of Fig. 3.

Referring again to Fig. 16, and using the same notation as before, let further

$\Delta =$ density of material of wire,

$\sigma =$ its specific heat,

$t =$ time from instant of making or breaking circuit.

Then before steady conditions have been established, let the rise in temperature of the element MN in the time δt be $-\delta\theta$. We shall then have to add to the right-hand side of equation (1) the term

$$A\,\delta x\,\Delta\sigma\left(-\frac{\delta\theta}{\delta t}\right),$$

to take account of the rate of heat storage in the element, and on reduction as before our fundamental equation becomes

$$KA\frac{\partial^2\theta}{\partial x^2}-A\Delta\sigma\frac{\partial\theta}{\partial t}-Ea\theta-\frac{i^2\rho}{A}=0. \tag{15}$$

Putting $m^2=(K/\Delta\sigma)$, $b^2=(Ea/A\Delta\sigma)$, this can be written

$$\frac{\partial\theta}{\partial t}=m^2\frac{\partial^2\theta}{\partial x^2}-b^2\left(\theta+\frac{i^2\rho}{EA^2a\Delta\sigma}\right). \tag{16}$$

This is a form of the Fourier equation of heat conduction, and the boundary conditions to be satisfied are:

(i) The heat conduction $\sum_{1,2} KA(\partial\theta/\partial x)$ along the wires away from the junction must have the constant value $-Pi$, independent of t, when $x_1=x_2=0$.

(ii) When $t=0$, θ must be zero for all values of x.

(iii) When $t=\infty$, the solution must tend to identity with (4).

It is not proposed to give the method by which the general solution of (16) is found (see Carslaw, 1921 or Ingersoll and Zobell, 1913). The final result is, however,

$$\theta=\frac{\lambda Pi}{2\sqrt{(KAEa)}}\left[e^{-\alpha x}\frac{2}{\sqrt{\pi}}\int\limits_{\{x/(2m\surd t)-b\surd t\}}^{\infty}e^{-\gamma^2}\,d\gamma-e^{\alpha x}\frac{2}{\sqrt{\pi}}\int\limits_{\{x/(2m\surd t)+b\surd t\}}^{\infty}e^{-\gamma^2}\,d\gamma\right]-\frac{i^2\rho}{EaA}(1-e^{-b^2t}). \tag{17}$$

Writing $x=0$, $\theta=\theta_0$, we get for the tip,

$$\theta_0=\frac{\lambda Pi}{\sqrt{(KAEa)}}\frac{2}{\sqrt{\pi}}\int\limits_0^{b\surd t}e^{-\gamma^2}\,d\gamma-\frac{i^2\rho}{EaA}(1-e^{-b^2t}), \tag{18}$$

the value of b^2 being $Ea/A\Delta\sigma$.

This result shows at once that the temperature of the junction at any instant is the resultant of two terms—one depending on the Peltier effect and following the error-function law, and the other due to the Joule effect and following a simple exponential law. The former term converges to its final value much more rapidly than the latter, and it is due to this fact that the 'humps' of Fig. 3 appear (see Fig. 18).

On breaking circuit after steady conditions have been established, the general

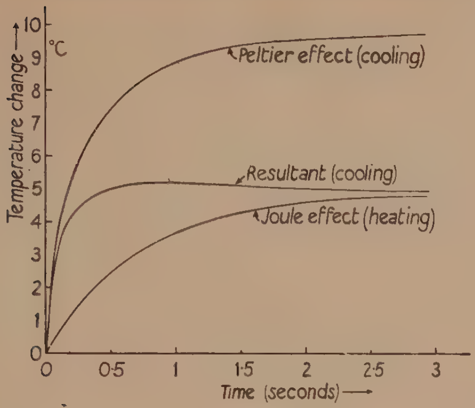

FIG. 18. Temperature change of thermocouple tip on starting current. $i = i_{\text{max}}$. Note: Couple in dry air. For details of thermocouple see text.

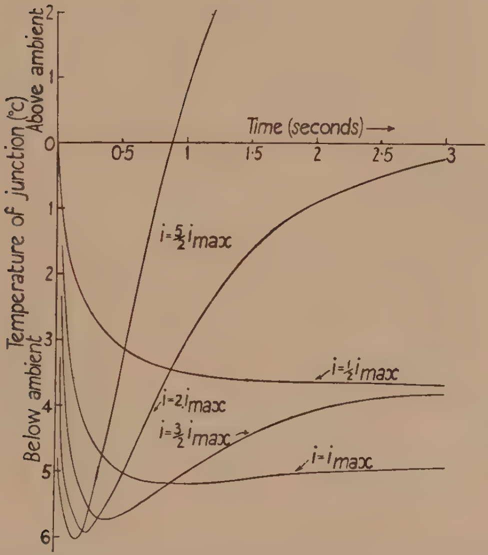

FIG. 19. Fall of temperature of junction on starting current. Note: Junction in dry air. For details of thermocouple see text.

equation (16) will apply with the term in i^2 omitted, and there will be new boundary conditions. These may be written:

(i) The heat conduction $\sum_{1,2} KA(\partial\theta/\partial x)$ from the tip must be zero for $x_1 = x_2 = 0$ for all values of t.

(ii) When $t \to \infty$, θ must tend everywhere to zero.

(iii) When $t = 0$, θ must have the form of equation (4).

The solution in this case (putting $x = 0$) is

$$\theta_0 = \frac{\lambda Pi}{\sqrt{(KAEa)}}\left[1 - \frac{2}{\sqrt{\pi}}\int_0^{b\sqrt{t}} e^{-y^2}\,dy\right] - \frac{i^2\rho}{EaA}e^{-b^2t}, \tag{19}$$

i being the current flowing prior to breaking the circuit.

As before, a concrete example will make matters clearer. Assume a couple of long bismuth and bismuth-tin wires of diameters 25μ and 34μ respectively. Then using the data of Appendix II, and taking the densities $\Delta_1 = \Delta_2 = 9{\cdot}80$ g. per cm.^{3} and the specific heats $\sigma_1 = 0{\cdot}122$, $\sigma_2 = 0{\cdot}133$ joules per g. per °C., the ratios λ_1 and λ_2 work out as 0·45 and 0·55, and the coefficients b as 1·29 and 1·06. Equations (18) and (19) are insufficient in so far as they assume that λ_1 and λ_2 do not vary with time. However, this is probably an insignificant point. Giving b the mean of its two values, and writing $\theta_{\text{max}} = 4{\cdot}9°$ C., equations (18) and (19) can be put in the forms

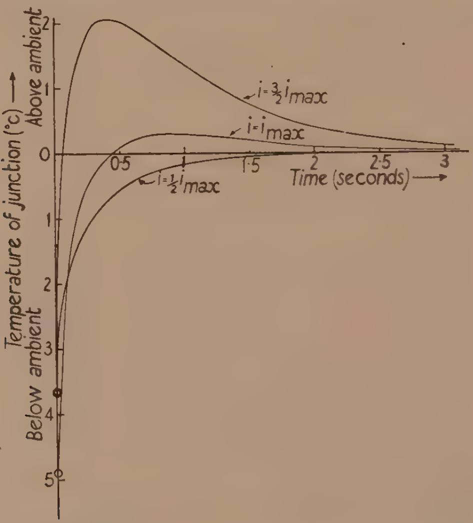

FIG. 20. Rise of temperature of junction on stopping current. Note: Junction in dry air. For details of thermocouple see text.

Making circuit: $\theta_0 = 9{\cdot}8(i/i_{\text{max}})\operatorname{erf} 1{\cdot}18\sqrt{t} - 4{\cdot}9(i/i_{\text{max}})^2(1 - e^{-1{\cdot}39t})$.

Breaking circuit: $\theta_0 = 9{\cdot}8(i/i_{\text{max}})(1 - \operatorname{erf} 1{\cdot}18\sqrt{t}) - 4{\cdot}9(i/i_{\text{max}})^2 e^{-1{\cdot}39t}$, where

$$\operatorname{erf} z = \frac{2}{\sqrt{\pi}}\int_0^{z} e^{-y^2}\,dy.$$

These two equations are used to plot the curves of Figs. 18, 19, and 20. They show clearly how the Peltier and Joule effects interact to produce the 'humps' of Fig. 3, and that these become more marked and occur sooner the greater the current. They also show that on breaking circuit the temperature of the tip undergoes a reversal, a phenomenon readily observable with the present apparatus.

With a couple of the size quoted the final tip temperature is nearly attained after 2 sec. with currents below the optimum. In practice this time would be rather increased due to an accumulation of metal at the junction. For couples of different sizes the time will vary directly as the radius of the wires.

APPENDIX II

TABLE I

Properties of some metals and alloys

Metal	Reference metal	Thermo-electric power at 0° C. μV per °C.	Peltier coefficient at 0° C. *P* millivolts	Thermal conductivity at 0° C. *K* watts per °C. per cm.	Specific resistance at 0° C. ρ microhm cm.	Product *K*ρ
Iron . .	Pb	+17·7	+4·83	0·62	8·53	5·3
Copper . .	Pb	+2·8	+0·76	3·88	1·64	6·4
Constantan .	Pb	−35·3	−9·64	0·22	49·0	10·8
Manganin .	Pb	+1·4	+0·38	0·21	45·0	9·5
Chromel-*p* (Ni 90%, Cr 10%) .	Pb	+28·5	+7·78	0·20	70·6	14·1
Platinum .	Pb	−1·8	−0·49	0·695	9·83	6·8
Pt+15% Ir .	Pt	+14·1	+3·85	0·23	26·9	6·2
Au+40% Pd .	Pt	−26·4	−7·21	0·38 (25° C.)	24·7	9·4
Antimony .	Pb	+35·6	+9·72	0·19	39·0	7·4
Bismuth .	Pb	−74·4	−20·3	0·084	106·5	8·9
Bi+5% Sn .	Pb	+34·0	+9·28	0·05	270	13·5
Sb+50% Cd .	Pb	+134 (50° C.)	+36·6	0·023	1,700	39·1

Note. The Peltier coefficient is calculated from the relation $P = T(de/dT)$.

TABLE II

Properties of some typical couples with long wires

Combination (1) (2)	Peltier coefficient millivolts	Degree of cooling θ_{max} °C.	Diameters of wires $\mu = 10^{-4}$ cm. (1)	(2)	Optimum current i_{max} milliamps.
Iron–Constantan .	+14·47	1·6	41 (= 48 SWG)	73	6·3
Pt Ir–Au Pd . .	+11·06	1·0	41 (= 48 SWG)	40	2·8
Antimony–Bismuth .	+30·0	6·9	25	35	3·0
Bi Sn–Bismuth . .	+29·6	4·9	34	25	1·5
Cd Sb–Bismuth .	+57·0	8·5	63	25	1·9
Manganin–Constantan	+10·0	0·6	41 (= 48 SWG)	42	1·7
Chromel-*p*–Constantan	+17·4	1·5	46	41	2·6

Note. The junction is cooled when the current passes from the second metal to the first. The value of *E*, the surface emissivity, has been taken as $12{\cdot}4 \times 10^{-4}$ watts per °C. per cm.^{2} (see *Inter. Crit. Tables*, 1st edn., vol. **5**, pp. 234–5).

ACKNOWLEDGEMENTS

Acknowledgements are due to the Department of Scientific and Industrial Research, under a grant from which the earlier part of this work was carried out, and also to Professor F. G. Gregory, F.R.S., for his stimulating interest and suggestions, without which the work would probably never have been done. Thanks are also gladly given to Mr. F. S. Smith for his help in the construction of apparatus.

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The Diageotropic Behaviour of Rhizomes

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SUMMARY

1. The geotropic behaviour of rhizomes of *Aegopodium podagraria* has been investigated by time-lapse photographic recording using infra-red radiation in complete absence of light.

2. They have been shown to be very sensitive to light. Even a single exposure for 30 seconds to red light evokes a characteristic response in which the rhizome first turns downwards and then turns up again to the horizontal position. The exposure to light during the process of digging up the rhizomes and setting up in the apparatus has similar effects which die away after about 24 hours.

3. After recovery from this initial light effect they continue to grow roughly horizontal if maintained in darkness or subjected to infra-red radiation only. If inverted (turned through 180°) the side which was lowermost during the inversion grows for about 2 hours relatively the more quickly, thus becoming convex; then a rapid straightening and bending in the opposite direction occurs. This is followed by further up-and-down movements which gradually die out and the rhizome then continues growing straight.

4. Inversion for 10 minutes also evokes an essentially similar response, which begins about 20 minutes after the inverted rhizome has been returned to its original position. The important theoretical significance of this is discussed in the text.

5. Displacement of rhizomes through angles other than 180° results in to-and-fro bendings in which the side of the rhizome which was uppermost before the stimulus of displacement first becomes convex. The rhizomes then turn gradually towards the horizontal plane.

6. Rhizomes which have recovered from the initial light effect and are thus growing in darkness horizontally are caused to turn upwards when the air surrounding them is replaced by air + 5 per cent. carbon dioxide.

7. The theoretical significance of these findings is discussed.

INTRODUCTION

EXPLANATIONS of the positive and negative orthogeotropic movements of plant organs have been advanced that have come to be regarded as satisfactory (see reviews by Rawitscher, 1932; Went and Thimann, 1937). These suppose that 'auxin' accumulates markedly on the lower side of a horizontally placed organ. The resulting unequal concentration causes an acceleration of growth of the lower side in stem structures and a corresponding retardation in roots.

The accumulation of an auxin on the lower sides of these orthogeotropic organs seems to be correlated with a potential difference between upper and lower surfaces such that the latter is positive in an external circuit (Brauner, 1927, 1928; Lund, 1947).

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It is clear that no simple extension of this type of hypothesis will form a satisfactory explanation of the phenomena of plagio- or diageotropism. The geotropic behaviour and the position taken up by plagiotropic lateral branches is generally regarded as a resultant of orthogeotropic and epinastic responses controlled by an auxin derived from the main apex (Münch, 1938; Snow, 1945).

There is no clear evidence of equality of hormone concentrations on upper and lower sides of plagiotropic organs in their equilibrium position. Nor are there clear-cut hypotheses of the mechanism by which displacement from the equilibrium position causes response.

Much of the work done (cf. Zimmermann, 1924; Rawitscher, 1932) was carried out before anything was known of the growth-controlling influence of trace metabolites and is expressed in terms that cannot be translated easily into those of metabolic or molecular reactions. Metzner (1929) invokes tonic influences on main and secondary geotropism (*Haupt-* and *Nebengeotropismus*) as the controlling agencies.

There is undoubtedly a considerable range of different types of plagiotropic behaviour found in organs of such different structure as lateral roots, lateral stems, petioles, leaf laminae, stolons, and others. The same is true of the special case of diageotropism where the organ assumes a horizontal position. Sometimes, as in Iris rhizomes, the organ is inherently dorsiventral, while in others, such as *Acorus*, the dorsiventrality (roots from lower side) is rapidly altered following a rotation of the rhizome into a new position. More commonly, however, rhizomes show a radial symmetry.

Many rhizomes appear to be genuinely diageotropic. Elfving (1880) and Czapek (1895) showed that *Eleocharis*, *Adoxa*, and other rhizomes planted in moist soil and placed sloping obliquely upwards or downwards develop new internodes which assume a horizontal position 10–14 days afterwards. Elfving showed that *Eleocharis* rhizomes planted upside down continued to grow horizontally without twisting, so their direction of growth was clearly not determined by an inherent dorsiventrality.

Plagiotropic organs behave differently from many diageotropic organs in that severing from the main stem or root usually causes them to become orthogeotropic. Horizontal or diatropic rhizomes generally maintain their behaviour for some time even when severed from the orthotropic and aerial parts of the plant.

Early discussions of diageotropism (cf. Czapek, 1895; Rawitscher, 1932; Stiles, 1936) were concerned with the problem of whether the phenomenon was caused by accurate balancing of an orthogeotropism by a curvature due to some other factor or was the result of diageotropism existing as a separate entity.

Raunkiaer (1907), indeed, has claimed that the depth of certain rhizomes was controlled by the length of the aerial shoot which was darkened by the soil, and Clapham (1945) regarded the direction of growth of *Polygonatum* rhizomes as due to a balance between negative geotropism and a downward curvature caused by light reaching a sensitive region possibly near the bases of aerial shoots. Such suggestions, even if correct, could have no general application as they would not explain the responses of portions of rhizomes detached from the remainder of the plant.

It was considered (Rawitscher, 1932) that the problem could be settled by the crucial experiment of rotating rhizomes obliquely at 45° to the axis of a klinostat of which the axis of rotation was horizontal. The orthotropic stimulation received during one half-rotation is reversed in the next half-rotation and consequently no curvature of the rhizomes would be expected towards the horizontal plane if their direction of growth was determined by the balancing of an orthogeotropic tendency by another factor. Czapek (1895) carried out this experiment and found that no curvature occurred, and it would seem that later investigators also failed to observe curvature of rhizomes fixed obliquely on a horizontal klinostat. The view is thus widely held (cf. Stiles, 1936) that the transverse position is maintained by balancing of opposing factors.

Some of the earlier investigators fully realized that not only does gravity exercise a controlling influence on growth of rhizomes, but that light also has an effect. Illumination of plagio- and diatropic organs was described as altering the 'geotonus' and causing downward bending, e.g. in *Ranunculus repens* (Zimmermann, 1927). Stahl (1884) had previously observed that the diageotropic rhizomes of *Adoxa* and *Circaea* and the plagiogeotropic lateral roots of *Vicia* all turned downwards on illumination.

The orientation of plagiotropic organs is also influenced by seasonal and internal factors. Vöchting (1898) and Lidforss (1903) have shown that at low temperatures in autumn, winter, and spring many otherwise orthotropic stems, *Lamium*, *Cerastium*, *Chrysanthemum*, and many others, become plagio- or diageotropic, and Schwabe (1950) has recently shown that in garden Chrysanthemum short days may induce development of diageotropism of aerial stems.

Excellent reviews (Rawitscher, 1932, 1937; Zimmermann, 1927) make it unnecessary to discuss the literature fully, but it is abundantly clear that detailed reinvestigation of certain geotropic phenomena is required.

Two points seemed to merit special attention at the outset. The supposedly crucial experiment of rotation of rhizomes fixed obliquely on the horizontal klinostat does not in fact cancel out the orthotropic stimulation unless one assumes the complete absence of 'longitudinal effect' (*Längskraft*) and a more careful repetition of this experiment seemed desirable.

Again, since orthogeotropic curvatures are considered to be caused by a transverse redistribution of an auxin, the possibility of such a redistribution in horizontal organs should be considered. If the 'auxin' contents of upper and lower halves of the horizontally placed rhizomes are unequal, inversion (a turn through 180°) might be expected to cause a curvature reaction capable of analysis. The investigation of these points is discussed in this paper, and this has also revealed a number of other features of the physiology of rhizome growth.

MATERIAL AND METHODS

Rhizomes of *Aegopodium podagraria* were available in large enough quantities and were our main source of experimental material; and in early trial experiments they, and also rhizomes of *Equisetum arvense*, *Lamium album*, *Calystegia sepium*, and *Agropyron repens* were examined. Rhizome apices were difficult to obtain in sufficiently large numbers in *Equisetum* and *Calystegia*; those of *Agropyron* grew very slowly after detachment.

Aegopodium rhizomes grow out in spring from the axils of both foliage leaves and scale leaves of the rhizomes of former years. The apical meristem is encased in and protected by a scale leaf which gradually enlarges as the internode pushes forward. On the cessation of growth of the first internode, the next internode commences to develop and some further slight enlargement of the scale leaf occurs. The elongation of the second and then of subsequent internodes proceeds similarly; it is characteristic that only one internode elongates at a time and a short 'dormant' period occurs just before a new internode is about to emerge. Several internodes can be produced during a growing season by a rhizome which is left attached to the main plant, and they maintain their horizontal course usually about 15–40 mm. below the soil surface.

Rhizome apices of *Aegopodium* may continue to grow horizontally apparently for an undefined number of internodes during any one growing-season from about March to November. In the following spring these apices produce aerial foliage leaves, and growing thicker and very slightly longer they turn into a vertical position remaining underground until eventually inflorescence axes develop from them.

A certain percentage of the rhizome apices change their behaviour during their first March–November growing-season in the same manner. The scale leaf emerges as a foliage leaf; the apex virtually stops elongating and becomes vertical and develops a crown of foliage leaves, remaining itself below ground until it forms an inflorescence.

Detached portions of rhizome continue to grow quite rapidly and at roughly constant rates for some 4–6 days after detachment and then with decreasing rate until about 14–20 days after detachment, when further elongation ceases and the terminal scale leaf develops a new form of meristematic activity forming a crozier-shaped foliage leaf with a negatively geotropic petiole. The emergence and photosynthesis of this foliage leaf is followed by the development from the apex of the rhizome of a sequence of further foliage leaves, and the apex enlarges somewhat and turns so that it points vertically, forming a subterranean vegetative apex as described above. It is an obvious assumption that loss of supply of certain metabolites brings about this change in behaviour.

Aegopodium rhizomes *whilst growing* appear to maintain their diageotropism and in this differ from, for example, *Lamium album* and *Calystegia*, where under certain conditions not yet analysed the rhizome apices lose their diageotropism and turn up as negatively orthogeotropic aerial stems. The biological consequences of this behaviour are closely similar to those following conversion of the rhizome apex of *Aegopodium* to a leafy vegetative apex; leaves are carried out into the light, but in *Aegopodium* this is effected by the negatively geotropic petioles.

One may note that certain rhizomes, such as *Mercurialis*, scarcely grow at all after detachment whilst others like *Aegopodium* grow rapidly.

The rate of growth of detached portions of *Aegopodium* rhizome is to some extent dependent on the length of the piece, as data in Table I show.

TABLE I

Elongation of detached portions of Aegopodium *rhizomes in water and 2 per cent. sucrose solution at 15° C. during the 24 hours following detachment*

Length (mm.)	Increase in water (mm.)	Increase in sucrose (mm.)
10	0·66	0·73
20	1·52	1·17
25	1·10	1·58
50	2·11	—
100	3·50	—
	4·94	
150	5·77	—

In the work which follows portions of rhizome 100–200 mm. long were used and preliminary experiments showed them to be very sensitive to displacement in the gravitational field. In the preliminary work they were fixed between sheets of wet filter-paper in chambers consisting of two glass plates held about 1 cm. apart. These were kept in a darkroom showing only slight temperature fluctuations and the angles of inclination of the rhizome apices to the vertical were measured by a protractor using a darkroom red or orange light, as earlier work already cited had made it clear that dia- and plagiogeotropism are influenced by light. Czapek and the earlier workers made their observations 5, 10, and 15 days after the gravitational displacement, but our observations were at first made at what we later came to regard as the relatively long time intervals of twice a day. The time course of the responses of rhizomes treated in this manner was studied in considerable detail In other experiments frames containing rhizomes placed obliquely were inverted at regular time intervals varying from 8 to 24 hours, so that the reversal of orthotropic stimulation as on a klinostat was obtained and these rhizomes also curved towards the horizontal plane.

In consequence, a klinostat was constructed which permitted more detailed study of this phenomenon regarded at one time as critical. Briefly, where the speed of rotation was once per 36 min. or longer, a slight but statistically significant curvature towards the horizontal took place within the first 24–48 hours.

We were at first inclined to regard these curvatures towards the horizontal which were obtained on the horizontal klinostat as of critical importance following the arguments of Czapek (1895) and Rawitscher (1932). We thought that our 'positive results' were obtained as a result of slower rates of klinostat rotation and possibly of making observations after much shorter periods of time than were used by Czapek. Later, as the data given in the experimental section show, this view has had to be modified drastically, and these klinostat experiments are now seen to be the result of a most complex array of stimuli and probably give little assistance to analysis of the mechanisms underlying diageotropism. We return to this briefly in the final discussion.

Photographic recording of rhizome growth and curvatures was then commenced, and in order to avoid complications of phototropic sensitivity and altered 'geotonus', the darkroom red light and infra-red sensitive plates were used. It seemed desirable to obtain these photographic records at regular and relatively frequent intervals and an automatic time-lapse device was constructed and fitted to the back of a camera. Certain details of construction are given below. It was some time before it was realized that the unexpected and peculiar results, which were obtained on using this photographic recording, were due to the remarkable sensitiveness of these rhizomes to red light to which (by analogy with phototropic organs) we had supposed them to be indifferent. The nature of the response is not phototropic, but is a change of geotropic sensitivity and will be dealt with below.

The recording apparatus to be described was therefore constructed so that rhizomes once set up could be subjected to various experimental treatments without exposing them to visible light; their growth changes were then recorded photographically using infra-red radiation to which they appear to be indifferent. Fig. 1 gives a simplified and conventionalized diagram of the apparatus.

Essentially, a camera, *C*, is fitted with a special slide holder, *D*, closed at the front by a metal plate with a narrow vertical slit, *sl*, and containing a plate holder, *pl.h.*, which carries two $3\frac{1}{4}$ in. × $2\frac{1}{4}$ in. infra-red sensitive plates. The threaded rod, *tr.*, driven by a motor, *e.m.*, through reduction gearing, and controlled by a relay operated by a clock (not shown), moves the plate $\frac{1}{4}$ in. at a time. A second relay operated by the same clock turns on the two 60-watt striplight lamps placed symmetrically in front of the windows, *w*, giving an exposure of about 2 min. Visible radiation and some of the shorter wavelength infra-red was screened off by fixing over each of the windows three layers each of Ilford filter No. 207 and three of No. 813. A light-tight tunnel connects the camera, *C*, with the light-tight box, *B*, containing the rhizome chamber, *A*; this latter consisted of a metal frame, $38{\cdot}5 \times 28{\cdot}5 \times 1{\cdot}25$ cm., with a glass back cemented to it. To this frame a glass front was luted with a vacuum grease after the rhizomes had been fixed in position. Their tips were threaded through a perspex carrier and all but a short terminal region about 1 cm. long was covered on both sides with wet filter-paper which kept them wet and maintained the atmosphere inside the chamber saturated. The chamber contained 14, and in the later experiments 20, rhizomes shown as *r* in the figure. Gas inlets and outlets to the chamber were provided, and a stream of air collected outside the laboratory and saturated with water was blown through the apparatus at rates of 10–30 litres/hour. A light-tight slide, *ds*.$_1$, gave access for the setting up of the rhizome chamber in the apparatus and a similar slide, *ds*.$_2$, gave access to enable the plates to be changed in the plate-holder. Since the plate-holder moved ¼ in. at a time, 24 photographs were taken on the pair of plates.

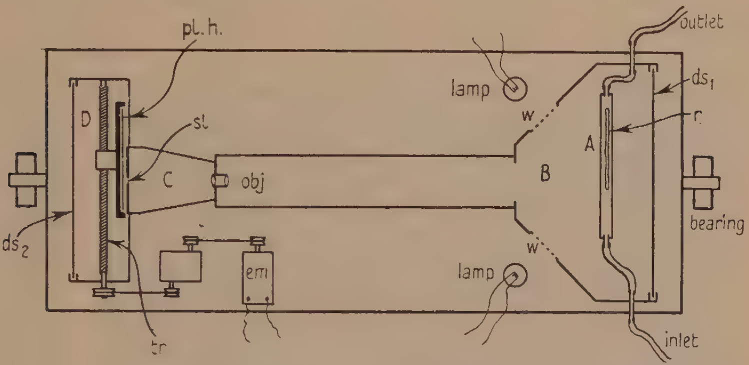

FIG. 1. Conventional plan of automatic photographic recording apparatus. For description, see text.

The whole apparatus was mounted on a rigid framework, shown diagrammatically, having bearings at the two ends. It was possible therefore to change the angle of inclination of the rhizomes at any desired time without any access of light. Movement of the whole framework in the direction at right angles to this was also possible, so that it was possible both to change the inclination of the long axis of the rhizomes to the horizontal plane and also to rotate the rhizomes around their longitudinal axes.

The type of record obtained by this apparatus is shown in Figs. 2 and 11. On the negative the rhizomes appear black against a white background on which are black lines 1 cm. apart obtained by fixing black paper with white ruled lines behind the back window of the rhizome chamber, *A*. Determinations of growth and of curvatures are made on the enlarged images of these negatives projected on a screen. The results of the first two seasons' work are not presented here, as the influence of red light was not realized at the time and although it is now possible to interpret some of the data, the design of these experiments in which geotropic stimuli are accompanied by unintended light stimuli is unsatisfactory.

A standard method of setting up was finally adopted; rhizomes were dug up, washed free of earth, and from a large batch 20 were selected. Those with slight crozier-like tips to the terminal scale leaf were rejected as they were most likely to fail to grow in length and were likely to be about to form negatively geotropic foliage leaves. A number of rhizomes curved rapidly in the first 2–5 minutes after release from the pressure of the surrounding soil and these were also rejected. Even so, the straight and uniform rhizomes chosen displayed considerable variability and sometimes up to 20 per cent.

of those chosen failed to grow and therefore proved completely non-responsive to gravitational stimuli; such non-growers were rejected when mean growth rates or growth curvatures were being computed.

The data presented in the following pages are a sample of many experiments the remainder of which are not quoted. Although there is marked variability amongst individual rhizomes due in part to unavoidable differences in the phase of growth of the extending internode, differences in diameter, &c., yet when the mean curvature reactions of batches of 20 are considered, remarkably similar behaviour is noted in replicated experiments. Individual rhizomes show considerable nutational movements, which are to some extent cancelled out in our records which give mean inclinations of the batch of rhizomes to the vertical plane. In citing these angles we have adopted the convention of terming the vertical down position as 0° (or 360°), vertical up as 180°, and horizontal, of course, as 90° or 270°.

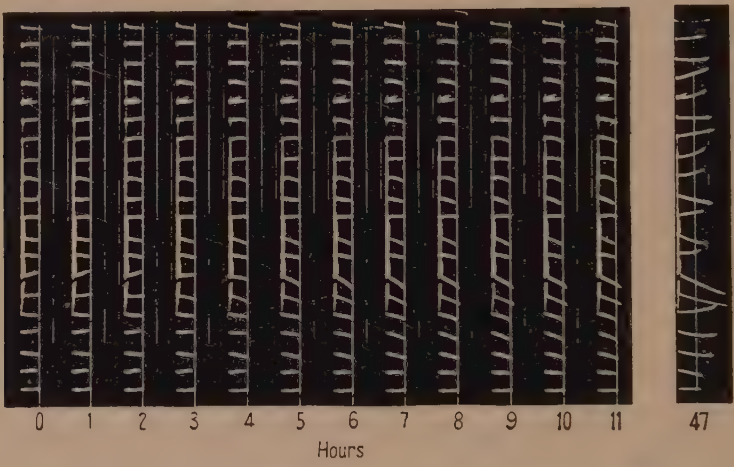

FIG. 2. Portion of the photographic record of Expt. 21/49 for period 0–11 hours from the start together with the final photograph 47 hours from the start. Results are plotted in Fig. 16. The vertical white lines are 1 cm. apart. The initial light response is illustrated by photographs 0–11 and the last photograph shows the position finally attained after 24 hours in the oblique down position.

We have no clear evidence of seasonal variation in sensitivity during the period May to September when most of the work described here was done, except for an apparent loss of responsiveness coupled with very reduced growth rates during parts of July and August. The cause of this has not been studied, but it has been noted that the phenomenon in each of three seasons coincided in time with flowering. In two of these seasons there was also rather prolonged drought, and either of these factors might be responsible for the considerable retardation of rhizome growth in July. Growth recommenced actively in mid-August and continued for a further 2 months or so before winter conditions again stopped it.

EXPERIMENTAL RESULTS

Effects of light on Aegopodium rhizomes

Experiment 2/48. A batch of rhizomes was dug, washed, and fixed in a position as close to horizontal as possible. Photographic recording was begun at once and, in this experiment, four 40-watt lamps covered with very dark red glass shades were employed as the illuminant and were placed symmetrically about 12 in. from the frame holding the rhizomes. Exposures for 30 sec. were repeated regularly every hour. The angles of inclination to the vertical plane were determined and the mean angles are plotted in Fig. 3. It will be noted that a slight upward movement in the first 2 hours is followed by a bending downwards until the angle with the vertical is 58°, 13 hours after the start; there is some slight recovery, but the rhizomes remain pointing downwards at about 15–20° below the horizontal.

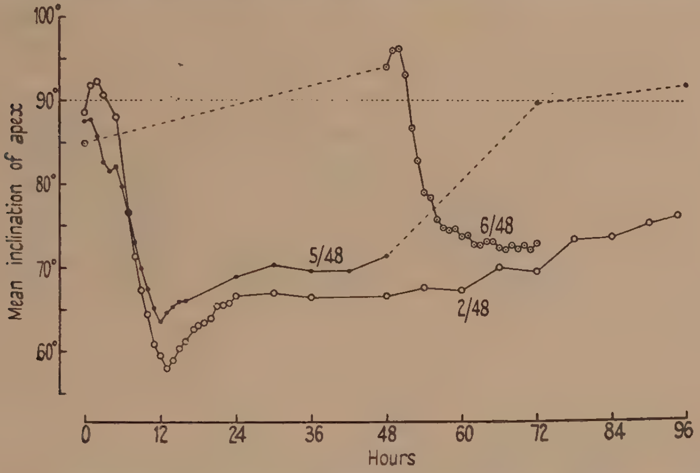

FIG. 3. Expts. 2/48, 5/48, and 6/48. Effects of intermittent red light on inclination of rhizomes to the vertical. From 24 to 96 hours photographs were taken hourly, but as change in inclination was small, the angles were measured at longer intervals as shown. Broken lines indicate periods of complete darkness uninterrupted by photographs.

Experiment 5/48 is essentially similar and the results are also plotted in Fig. 3. The downward curvature to about 70° (from vertical) follows the same time course. In this experiment the automatic recording was stopped at the 48th hour and the rhizomes were left in darkness for 24 hours and were then again photographed and are seen to have returned to their normal position of about 90°.

Experiment 6/48, also recorded in Fig. 3, was set up in the same manner, but the rhizomes were left in continuous darkness for the first 48 hours without any automatic recording; this process was then started, a 30-sec. exposure being given every hour. It will be noted that a rapid fall from about 92° to 72° takes place during about 16 hours.

The intermittent red light used in these recordings clearly causes the rhizomes to bend downwards from their normal position at 90° to about 70°. It should be emphasized that the light used strikes the rhizomes from the side and they bend downwards at right angles to the plane of the incident light. There is no phototropic bending towards or away from the light which operates by changing the geotropic response. Other experiments showed that when the visible red light was screened off by a suitable thickness of Ilford filters 207+813 which transmit infra-red, the rhizomes maintain their normal 90° position. Accordingly experiments were carried out to show how rhizomes behaved in darkness and following short single exposures to light.

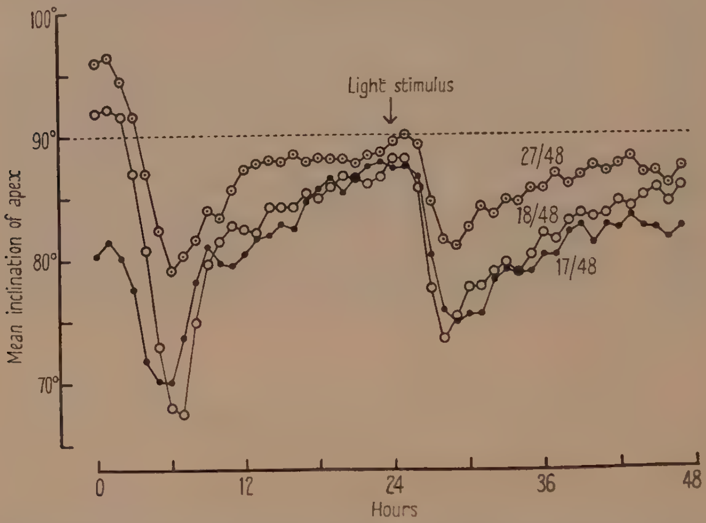

FIG. 4. Expts. 17/48, 18/48, and 27/48. Effects of 6 min. exposure to red light in 17/48 and 18/48 and 30 sec. exposure in 27/48. All recording carried out by infra-red radiation.

Experiments 17/48, 18/48, and 27/48. In each of these experiments batches of 14 rhizomes were set up in the usual manner and photographic recording was begun at once, but now the visible red radiation was screened off as described. The records are given in the same form as before in Fig. 4, which shows as ordinates the mean angles taken up in each batch of rhizomes. After an initial slight rise, the rhizomes dip downwards, attaining their minimum angle 6 or 7 hours after the start; they then recover fairly rapidly and have almost reached the normal position of 90°, 24 hours after the setting up of the experiments. At this point in Expts. 17/48 and 18/48 the screens were removed and the rhizomes were exposed to the weak red light for 6 min. and in Expt. 27/48 they were similarly exposed for 30 sec. The screens were replaced and photographic recording using infra-red radiation only was recommenced.

The effects of these short light exposures are made clear in the graphical records. After a 'latent period' of about 2 hours, the rhizomes bend downwards rapidly, and after a further 3 hours approximately they start to recover and gradually return to roughly the normal 90° position. The minimum angle attained after the 6-min. illumination is about 75° and after the 30-sec. flash it is about 81°. The relatively bright daylight and fairly long continued illumination to which they are subjected when dug up, washed, and finally fixed in the rhizome chamber produces an essentially similar initial reaction having much the same time course, and it will be noted that the minimum angles attained vary from 80° in Expt. 27 to 67° in Expt. 18. The fact that these very different dosages of light produce rather similar effects would seem to indicate that an effect close to the maximum is produced even by the short exposure of Expt. 27/48.

Other experiments not recorded here have shown in a similar manner that short exposures to green and violet light produce reactions essentially similar to those produced by red, but no attempt has yet been made to compare the quantitative efficiencies of the lights of different wave lengths.

Since the digging and fitting of the rhizomes in the apparatus inevitably involves an exposure to light, it is now clear that if one is to obtain a record of response to gravitational stimuli uncomplicated by this effect of light on the geotropic sensitivity, one must set up the rhizomes and leave them in darkness for at least 24 hours to recover from this initial light effect.

Effects of inversion of the rhizomes

Experiments 4/49 and 24/49 are two typical experiments of this type; in each case a batch of 20 rhizomes was set up as described and growth behaviour was recorded photographically using infra-red radiation only. The graphical record of Expt. 4/49, in which photographs were taken at 1-hour intervals, is given in Fig. 5. It will be noted that the initial light effect developed rather slowly and the minimum angle was attained about the 12th hour; recovery to the normal angle of 90° was not quite complete by the 23rd hour. At this point the whole apparatus was turned round through 180° so that the rhizomes were turned upside down. The graph shows that the mean inclination of the batch at this point was 85° (slope of 5° downwards), so that inversion converts this to an inclination of 95° (slope of 5° upwards). The immediate effect of the inversion is to cause a rapid upward bending movement which is due to the more rapid extension growth of the lower side of the rhizome, i.e. that side which had been uppermost before inversion. After this rapid upward bend, an equally rapid bend down occurs followed by a series of further bendings up and down of decreasing amplitude, until finally the direction of growth settles down at approximately the normal angle of 90°. At the close of this series of up-and-down movements the rhizomes are approximately straight.

The periodicity of these up-and-down movements varies somewhat from rhizome to rhizome and, in consequence, when the movements of individual rhizomes are out of phase, the mean amplitude of fluctuation is much less than that of individual rhizomes.

Owing to the rapidity of the movements, the photographs in Expt. 24/49 were taken at 15-min. intervals for 4 hours immediately after the time of inversion; the design of the experiment was otherwise exactly similar to the last and the results which are given in Fig. 6 are essentially similar. The short times between exposures enable one to see with greater precision the existence and duration of a short latent period before the curvature reaction begins. A special test experiment showed that 25 min. continuous exposure to infra-red radiation of the intensity used for the photographic exposures had no effect on the direction of rhizome growth, so the increased amount of this radiation needed for the 15-min. photographic records must have been without influence.

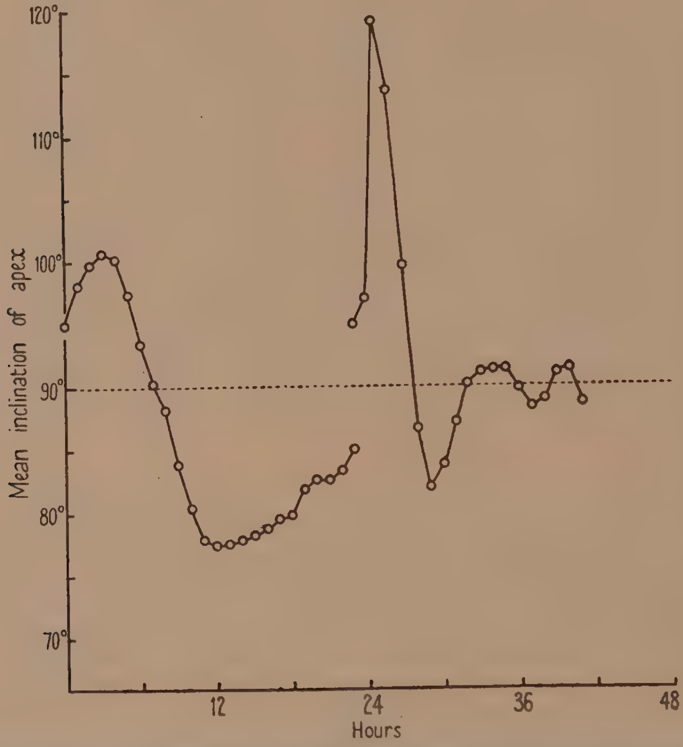

FIG. 5. Expt. 4/49. Effect of inversion of rhizomes at 23rd hour after placing in darkness in apparatus. Recording by infra-red radiation.

Experiment 23/49. This was set up in exactly the same way as the last two Expts. 4/49 and 24/49 and the course of change in angle during the first 24 hours is seen from Fig. 7 to be practically the same. At the 24th hour, when the mean angle was about 80°, the apparatus was inverted for 30 min., thus making the angle of the rhizomes about 100° during this half-hour period. After this half-hour period in the inverse position, the frame was turned back into its original position, and so, since little movement of the rhizomes had occurred, their angle was about 80° again. In the next 2 hours very rapid

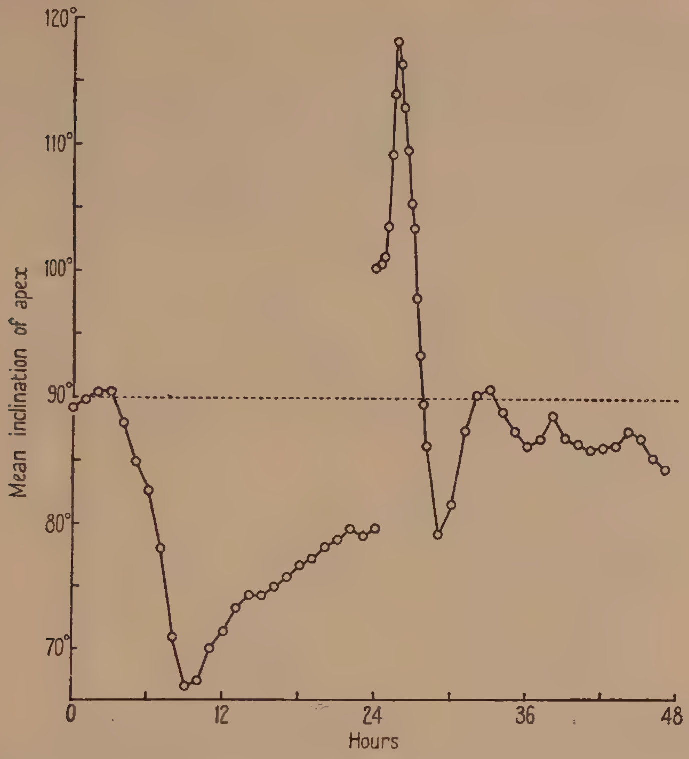

FIG. 6. Expt. 24/49. Treatment the same as Expt. 4/49 (Fig. 5) but with inversion at the 24th hour and photographs taken every 15 min. from 24th to 28th hour.

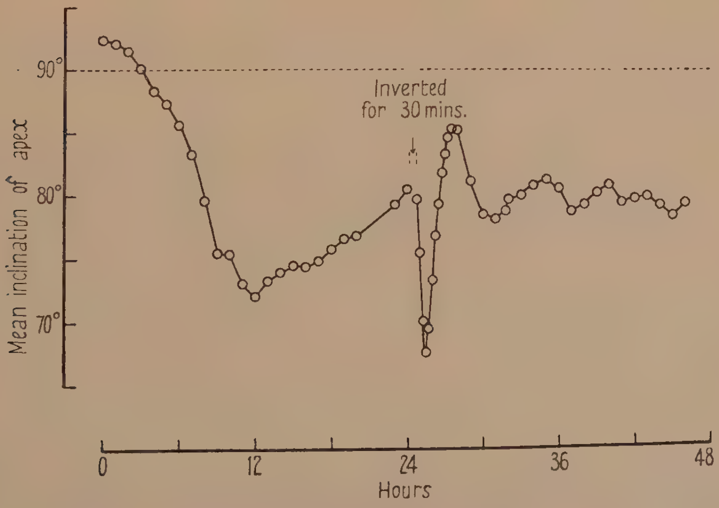

FIG. 7. Expt. 23/49. Rhizomes inverted at 24th hour for 30 min. Readings every 15 min. from then until 28th hour followed by usual hourly readings.

bending *downwards* occurred followed by a series of bendings up and down like damped harmonic oscillations.

The first visible response is that the side which is lowermost during the stimulus becomes convex owing to its extension being more rapid than that of the other side. This state of affairs is soon reversed and a series of reversals causes the up-and-down movements. The sense of the first curvature is the same as with continued inversion, where the side lowermost immediately after inversion becomes convex at first, and then the sequence of oscillations follows. Thirty minutes inversion produces nearly as large a reaction as continued inversion.

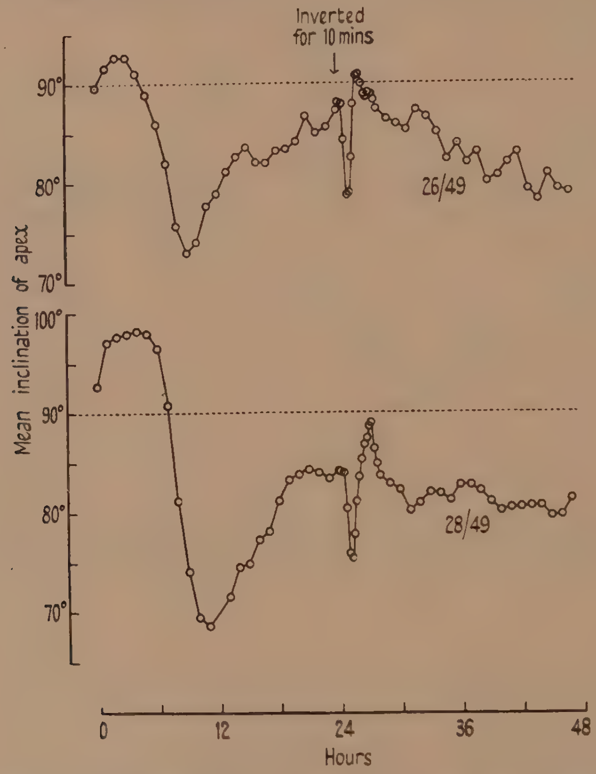

FIG. 8. Expt. 26/49, upper record, and Expt. 28/49, lower record. Rhizomes inverted for 10 min. from hour 24.02½ to 24.12½. Photographs at hours 24.00, 24.15, and then at 15-min. intervals till hour 28.00. Hourly readings after that.

Experiments 26/49 and 28/49, illustrated in Figs. 8 and 10, are essentially similar to the last. Batches of 20 rhizomes were allowed to recover as usual from the initial light effect for 24 hours. They were then inverted for 10 min. and turned back into the original orientation; the stimulus began 2½ min. after the 24th-hour photograph and the next photograph was taken 15 min. after the 24th hour, i.e. 2½ min. after the rhizomes had been returned to their original orientation. The response evoked is essentially as before, in that the side which was lowermost during the stimulus of inversion first of all becomes convex. This is followed by the usual up-and-down oscillations, though their amplitude is rather smaller than those which follow the 30-min. or continued stimulation. The important point to which we return in the discussion is that during the 20 min. following the 10-min. stimulus little or no movement occurs; very rapid bending then follows.

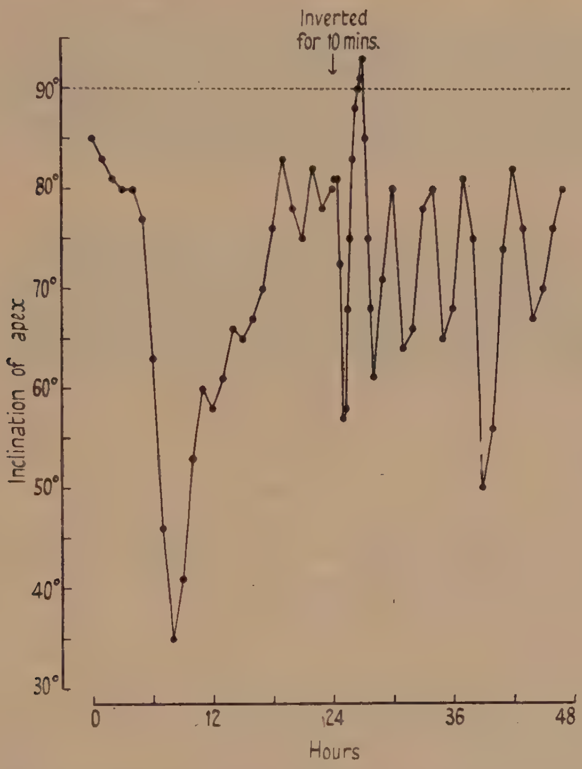

FIG. 9. Record of behaviour of one active rhizome, No. 16, from Expt. 28/49, for which the mean inclinations are given in Fig. 8. Inversion from hour 24.02½ to 24.12½.

The graphical record of Fig. 8 which gives the mean inclination of the whole batch of rhizomes makes these points clear. The behaviour of one single very rapidly growing rhizome is illustrated separately in Fig. 9. One should note that this individual bent down to an inclination of 35° compared with the mean 68° during the response to the initial light stimulus. The inversion stimulus caused a 24° deflexion as compared with a deflexion of about 9° when the mean is taken. Up-and-down oscillations of 15–40° occurred for at least 24 hours after the stimulus.

When mean inclinations are plotted, comparatively little change is recorded as the time course of the movements differs slightly from individual to individual and the crests and troughs to some extent cancel each other.

The detailed time course of the mean inclination and of this particular rhizome are again shown in Fig. 10 on an enlarged scale between the 21st and 30th hour. The stimulus of inversion lasted from $2\frac{1}{2}$ to $12\frac{1}{2}$ min. after the 24th hour, and the record shows clearly that no response has occurred at the second reading, i.e. $17\frac{1}{2}$ min. after the rhizomes had been returned to their original orientation. Part of the photographic record of this experiment is reproduced as Fig. 11.

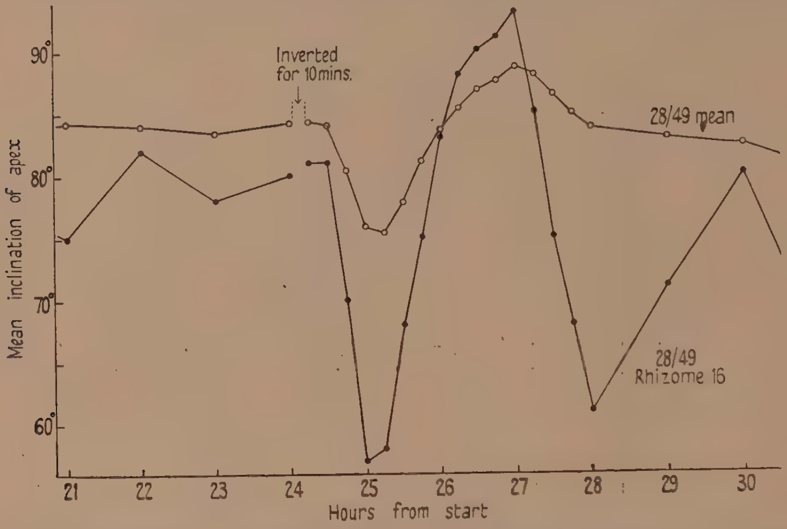

FIG. 10. Part of the record of Expt. 28/49 on enlarged time scale to show details. Mean inclination for batch shown –○ –, that of rhizome No. 16 shown – ● –.

These two experiments, 26 and 28/49, show another feature with particular clearness; during the first 2–3 hours after the start of the experiments, the rhizomes which had been set in the apparatus at approximately 90° turned up a few degrees and then began to turn down showing the typical light effect. This initial turning up is of frequent occurrence, although it is not always observed, and in some experiments the upward deflexion is non-significant. It seems probable that it is in fact the response to an inversion stimulus; since these rhizomes are radially symmetrical, one cannot at all readily tell which was the upper side in the ground, even with very careful and slow digging out, and it has been our custom to set them up without any reference to their former orientation in the soil. Consequently, some of those set up are bound to be put in upside down and to give the inversion response which we have just seen is a turning upwards within an hour; others will have been rotated about their horizontal axes to different extents and this treatment produces similar responses as the next section will show. The onset of the light responses, a turning downwards, is rather slower, starting usually 2–3 hours after the stimulus.

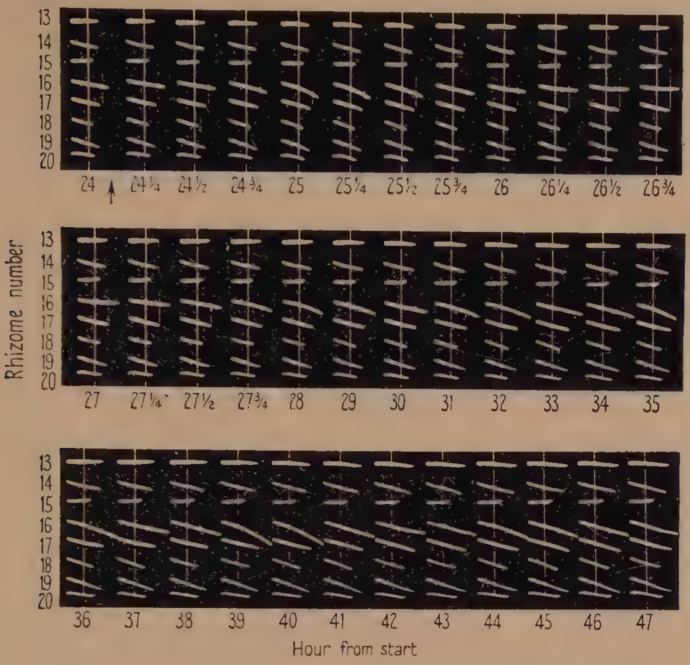

FIG. 11. Expt. 28/49. Portion of the photographic record showing the movements of rhizomes 13–20 between the 24th and 47th hours. Just after the 24th hour, at the point indicated by the arrow, the rhizomes were inverted for 10 min. No. 16 rhizome, which was growing rapidly, shows very active movements. The movements of this rhizome have been plotted separately in Figs. 9 and 10, and the mean movement in Figs. 8 and 10. In calculating the means, the values for rhizome No. 15, which shows practically no elongation, have been omitted.

The effects of rotation about the longitudinal axis

Experiment 5/49. The rotation stimulus was effected as follows: the usual batch of 20 rhizomes was set in the frame which was fixed in the automatic camera and the whole apparatus was then turned into the vertical position so that the rhizome frame lay horizontal and the rhizomes were photographed from above. The initial bending downwards of the rhizomes due to the light effect took place in a plane parallel to, not perpendicular to, the optic axis of the camera. During the first 24 hours the photographs record the almost negligible side-to-side nutations of rhizomes which are carrying out the down-and-up light reaction in the plane at right angles. The record is given in Fig. 12.

At the 24th hour the apparatus was replaced with the camera long axis again horizontal and the rhizome frame of course vertical. The photographs now show up-and-down movements of the rhizome and it will be seen that the response is almost the same as that evoked by inversion; the side lowermost on stimulation becomes convex at first, followed by the usual oscillations.

Fig. 20 will make clear the nature of the experimental displacement of the rhizomes which we are describing as a 90° rotation.

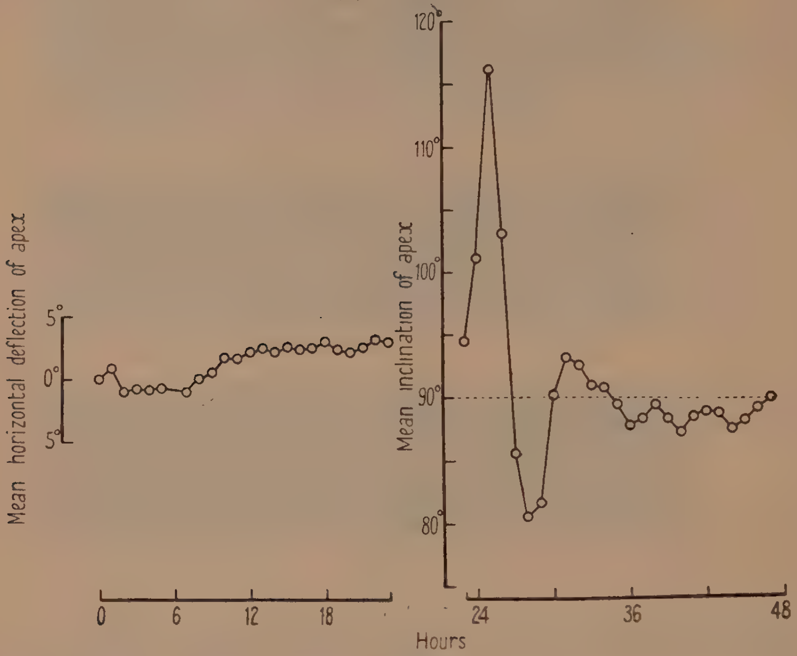

FIG. 12. Expt. 5/49. Effect of rotation through 90° around the longitudinal axis of the rhizomes. For details, see text.

The effects of vertical orientation

Experiment 7/49. After the usual 24-hour recovery period, the frame containing the batch of 20 rhizomes was turned through 90° in a vertical plane. The results are recorded in Fig. 13, where it will be noted the ordinates extend from 60° to 115° on the left half of the record and from 155° to 205° on the right half. The left half of the record shows the mean inclinations during the first 24 hours, the rhizomes having been set up horizontal and naturally responding in the usual way to the light stimulus received on being dug up. At the 24th hour they were turned so as to point up (180°) and the ordinates are taken so that the 180° position is now in the middle. The arrows on the two portions of the record indicate the positions of the rhizome-containing frames. The immediate effect of the stimulus after a short latent period is to cause a rapid drop in angle; in other words, the side of the rhizomes which was uppermost before the displacement to the vertical becomes convex.

After this first downward movement, which is carried out by all the rhizomes, there is a striking diversity of behaviour. A few individuals (in this experiment, 4) turn back again into the 180° position, becoming quite straight, and then continue to bend over, turning downwards in such a way that the side uppermost before the stimulus is concave. These angular deflexions are recorded as larger than 180° and the final horizontal orientation would of course be 270°. Other rhizomes (in this experiment, 14; 2 out of the 20 failed to grow) do not turn completely back to the original vertical position after the first rapid phase of response to the stimulus. About 12 hours after the rhizomes were put pointing upwards, 4 had curved with the original upper side concave and 14 with this side convex; a general mean angle is thus misleading, but it is plotted in the figure for reference together with separated means of the convex and concave series. Other experiments which are not quoted here owing to lack of space gave similar results, and make it clear that rhizomes eventually turn back from the metastable vertical up position with the original upper side either convex or concave, but the first movement following the stimulus is always due to the relatively more rapid extension of the original upper side, which thus becomes convex.

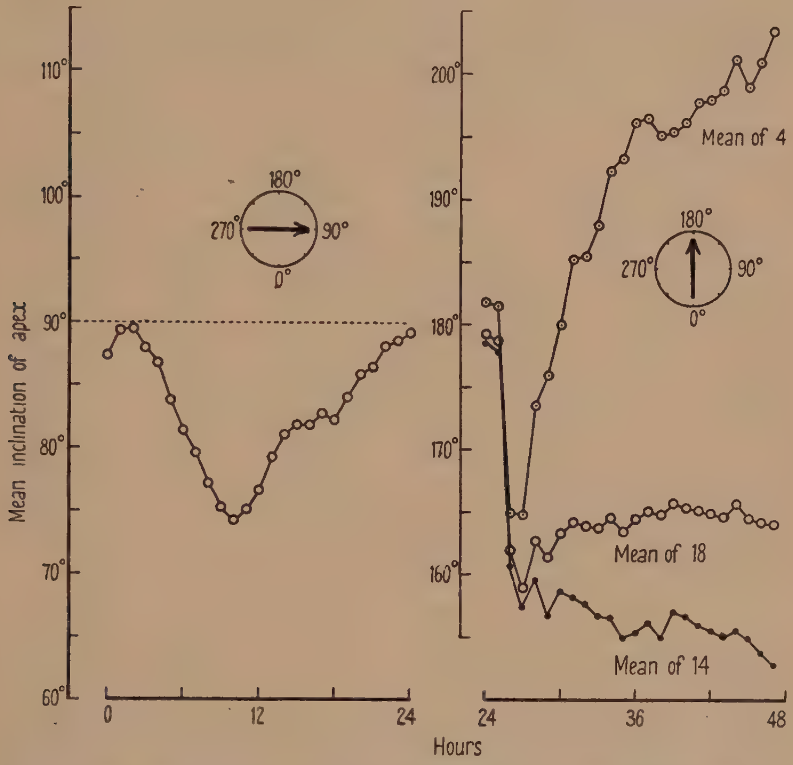

FIG. 13. Expt. 7/49. Effect of turning rhizomes into the vertical position pointing up. Treatment is shown by dials with arrows. For further details, see text.

Experiment 6/49. This experiment was similar in design to the last described but differed in that, after the turn through 90°, the rhizomes were pointing vertically downwards. The response shown in the records of Fig. 14 indicates again that after a short latent period a very rapid bending of the rhizomes towards the horizontal occurs in such a way that the side uppermost before the stimulus becomes convex.

In the graph the left half gives the mean inclinations during the first 23 hours, at which point the rhizomes were turned through 90° into the vertical down position (0° or 360°). As they then turn farther in the same direction their inclination is given as 328° at the time when it deviated farthest from the vertical; in other words, a deviation of 32° from the vertical is found. This is reached after 2–3 hours and then all the rhizomes start straightening out again quite rapidly; finally, about 6–8 hours after the displacement into the vertical down position, the rhizomes start slowly bending towards the horizontal position. In this experiment 9 turned over so that the former upper side was convex and 9 so that it was concave. In general, as in the case where the rhizomes were put pointing upwards, the majority tend to turn in such a way that the former upper side is convex, but some straighten out completely and turn in the opposite direction.

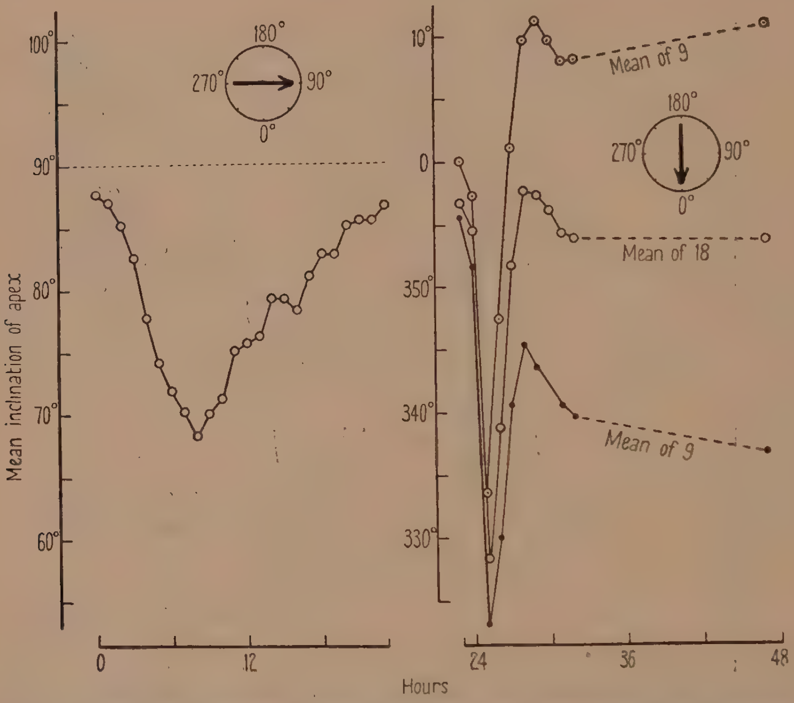

FIG. 14. Expt. 6/49. Effect of turning rhizomes into the vertical down position. Conventions as in Fig. 13. Owing to failure in the recording mechanism no photographs were obtained between 32nd and 47th hours.

In this figure, as in the last, the general mean angle of the whole set and the separated means of the convex and concave groups respectively are shown.

As will be pointed out more fully in the discussion, this rapid initial bending followed by the reverse movement is very similar in nature, extent, and time course to the movements that result from inversion or rotation: they are followed in the case of vertically placed rhizomes by a very slow curvature towards the horizontal plane. This latter is the strict diageotropic reaction, and for convenience we are terming the initial oscillatory reaction 'the displacement effect', as it seems to be evoked by any displacement in the gravitational field. In regard to the slow diageotropic reaction, we have not carried on the photographic recording long enough to observe full recovery to the horizontal from the vertical plane, but segments of rhizome planted vertically in soil certainly grow new internodes which can attain the horizontal plane.

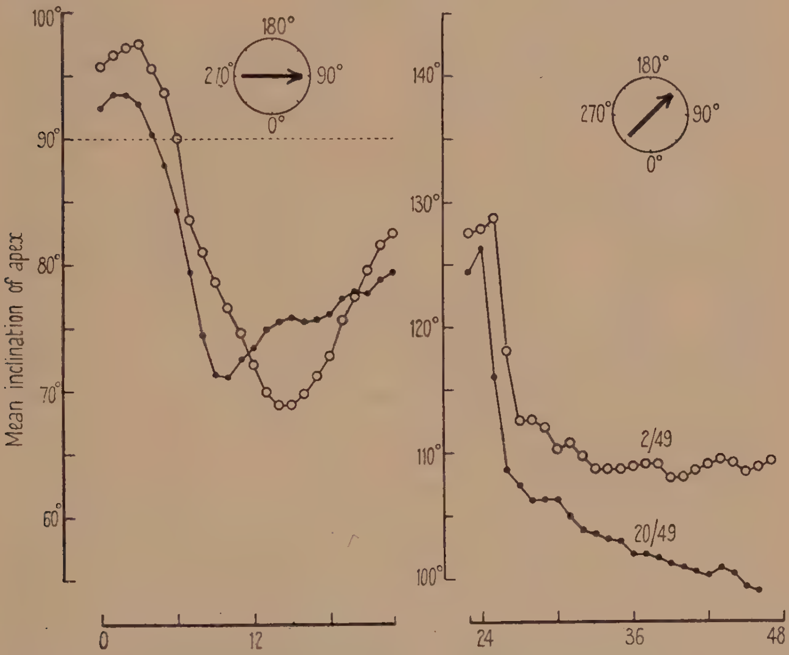

FIG. 15. Expt. 2/49, –○ –, and Expt. 20/49, – ● –. Effects of turning rhizomes into the oblique up position. Conventions as before.

The effects of oblique orientation

Experiments 2/49 and 20/49. In these two experiments, which are typical of a number, the method of setting up and presentation was essentially the same as for the last two described. After 24 hours to recover from the initial displacement and light effects, the frame was in each case turned through 45° so that the rhizomes pointed upwards, but, as they had not completely come back to the 90° position before displacement, the initial angles after displacement were only about 124° and 128° respectively. The response, which is shown in the records of Fig. 15, shows after a short latent period a very rapid initial bending in which the side uppermost before displacement becomes convex; this brings the angle to about 110° and from there a slow return towards the horizontal occurs.

Experiments 3/49 and 21/49 are an essentially similar pair of experiments in which the position of the rhizomes was changed at the 24th hour so that they pointed obliquely downwards. The initial angles immediately after displacement are seen from the record in Fig. 16 to be about 34° and 39°, and the first stage of the response is seen to consist of a further turning downwards of the rhizomes so that angles drop to a mean value in both experiments of about 29°.

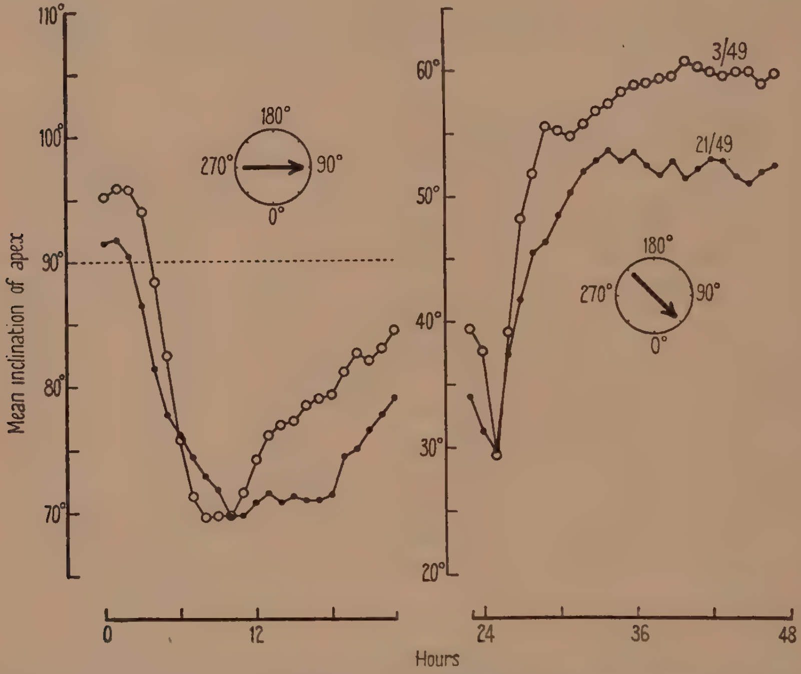

FIG. 16. Expt. 3/49, –○ –, and 21/49, – ● –. Effects of turning rhizomes into the oblique down position. Conventions as before.

In other words, in the first stage of the response the former *upper* side becomes *convex*, which it will be remembered happens with displacements to the angles of 0°, 180°, 135° as well as to 45°. In this last case this is followed by a sharp swing in the reverse direction like that found following the 0° and 180° displacements. This back swing brings the rhizomes well past their original oblique orientation to an angle of around 50° and from then on they slowly turn towards the horizontal.

Experiment 13/50 was set up as usual, and when the initial light effects had died out and the rhizomes were approximately again at 90°, the frame was turned through 135° so that the rhizomes were left pointing upwards at an angle of 45° to the horizontal in such a way that their upper sides were those which had been the lowermost before the stimulus. The positions of the frame are made clear by the arrows in Fig. 17. The oblique up position, immediately following the stimulus, is given, following our convention, as 225°. The immediate response is, as before, a rapid extension of the former upper side which thus becomes convex, causing the rhizomes to turn first towards the vertical (180°). Then there is a rapid swing in the opposite direction and past the 225° position, so that after straightening, the former upper side now becomes concave, and after a number of lesser oscillations the tendency for curvature towards the horizontal (270°) becomes apparent.

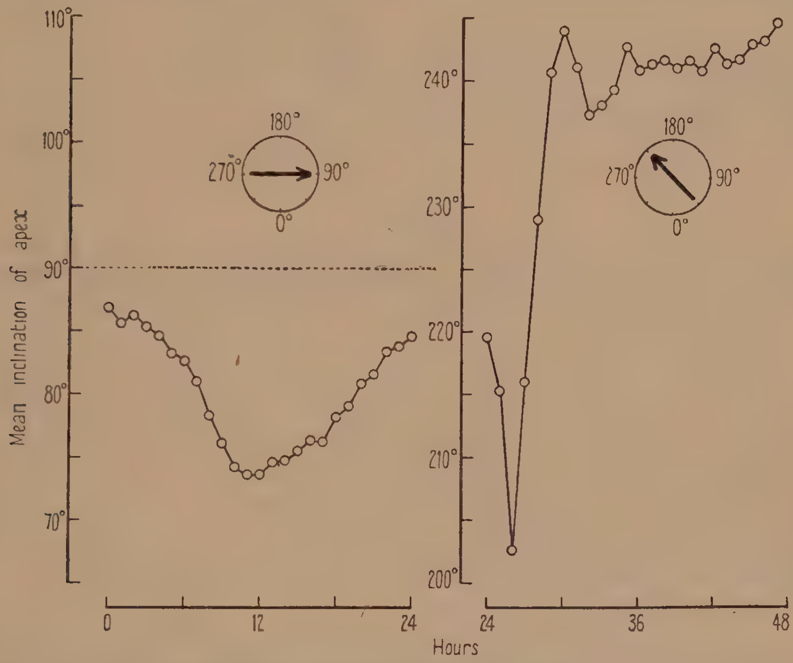

FIG. 17. Expt. 13/50. Effects of turning rhizomes through 135° into the oblique up position. Conventions as before.

The effects of carbon dioxide

Experiments 5/50 and 6/50 were set up in the usual manner with the rhizomes at an initial mean angle of 90°. Air from outside the laboratory was drawn through as usual at rates in these experiments of about 30 litres per hour. This air-stream was led through for 48 hours and, as will be seen in Fig. 18, the rhizomes in Expt. 6/50 have completely recovered from the initial light effect by the 27th hour and in Expt. 5/50 by the 30th hour. From the 30th till the 48th hour they grow straight at a mean inclination of about 86°. Immediately after the 48th-hour reading the air-stream was replaced by a 30 l./hour stream of air enriched with 5 per cent. CO_2.

In Expt. 6/50 there is a 2-hour latent period followed by a rise in inclination from 86° to 96° followed by a return curvature so that the rhizomes again become approximately horizontal. This is succeeded by a slow rise in angle of inclination. Finally, from about the 30th hour onwards after the supply of the 5 per cent. CO_2, a new equilibrium inclination at an angle of about 101° is attained. The final reading was 101°±1·81.

Expt. 5/50, which was not continued for so long, appears to be showing similar features.

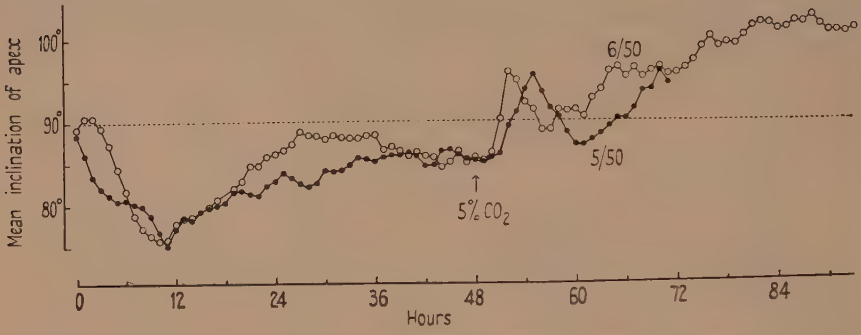

FIG. 18. Expts. 5/50 and 6/50. Rhizomes kept in darkness in an air-stream for 48 hours which was replaced (at arrow) by stream of air enriched with 5 per cent. carbon dioxide.

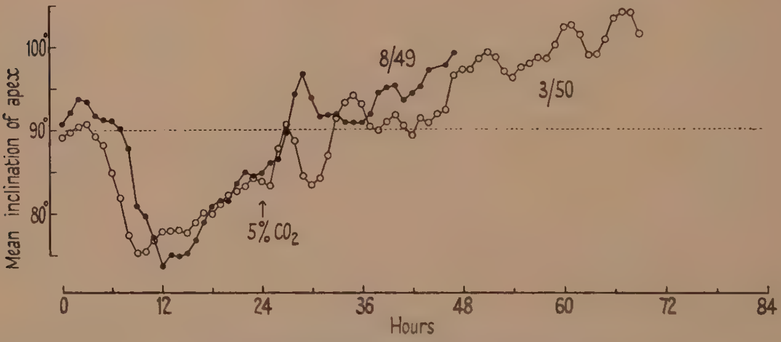

FIG. 19. Expts. 8/49 and 3/50. Treatment similar to that in Fig. 18, but carbon dioxide containing air-stream supplied at the 24th hour.

Experiments 8/49 and 3/50, the records of which are shown in Fig. 19, are similar to the two just quoted. They differ in that the 5 per cent. CO_2 was supplied immediately after the 24th-hour reading and possibly slightly before attainment of the equilibrium inclination in air. After a latent period, there is again a relatively rapid rise in inclination followed by a fall, a subsequent rise, and particularly in Expt. 3/50 a series of up-and-down movements. The general trend is towards a mean equilibrium inclination a little over 100°. The last reading was 101·5°±1·45°.

In other experiments not recorded here, similar results have been obtained using 5 per cent. CO_2. The effects of 10 per cent. CO_2 have also been studied, but the detailed results which are not presented were not significantly different from those with 5 per cent. CO_2. No significant difference in behaviour of the rhizomes in CO_2-free and in ordinary air has been observed.

Rhizome behaviour in other species

Space precludes full citation of experimental results with rhizomes of other plants, and we record very shortly here our finding that rhizomes of *Circaea lutetiana* show a light effect and displacement effects which appear to be essentially similar to those of *Aegopodium*.

Calystegia sepium and *Aster novae-angliae* (garden variety of Michaelmas daisy), on the other hand, showed no light effect in autumn. Displacement through 180° did not produce the characteristic oscillatory up-and-down movements characteristic of *Aegopodium* and *Circaea*. The detached rhizomes tended, moreover, to set at an angle between 100° and 120° as compared with the 80° to 90° commonly found in *Aegopodium*. When growing in soil and attached to the parent plant, they can extend for long distances almost horizontally.

DISCUSSION

It will be convenient to summarize very shortly the main experimental findings for which one wishes to understand the underlying mechanism.

(*a*) *The light effect*. Rhizomes of *Aegopodium*, *Circaea*, and probably many others of similar general structure are remarkably sensitive to very small dosages of light. In contrast to phototropism, the red end of the spectrum is fully effective. The direction of incidence of the light appears to be without influence and the effect produced is to increase the positive geotropic tendency of the rhizome which turns downwards. The effect of quite a large quantity of light, such as 30 min. exposure to bright daylight, gradually disappears when the rhizomes are placed in darkness, and has almost completely gone after 20–30 hours. Exposure to a weak darkroom red light for 30 sec. can then produce a reaction, as seen in Fig. 3, not very markedly less in extent than that evoked by the much brighter and longer continued light.

The effectiveness of very small quantities of light suggests that the response is initiated by the trigger-like action of some substance formed or changed very rapidly under the influence of light. One may compare the effectiveness of these small light doses with those which, given in the middle of the dark period of a photoperiodic cycle, eliminate its effectiveness in inducing flowering in a short-day plant such as *Kalanchoe* (Harder, Gummer, and Gall, 1945). Certain features of the light effect are worth noting in detail: a 'latent period' of about 2 hours following a 30-sec. exposure to light precedes a fairly rapid bending downwards which continues for another 2–4 hours. The recovery which follows begins relatively rapidly and becomes progressively slower, taking some 12–16 hours altogether. These movements, it should be noted, are much less rapid than those which follow the stimulus of inversion where the 'latent period' is of the order of 20 min. and the first deflexion movement is completed in some 30–45 min., the return movement (recovery) in the opposite direction takes about 60–90 min. and is followed by a series of harmonic oscillations of the same time course.

We do not at present know the effects of these light flash stimuli on cell numbers, nor do we know the extent of the apical region in which the stimulus is received. Speculation on the molecular mechanism is therefore at present not very profitable; it is of great importance to recognize, however, the extent to which the normal dark geotropic reactions are modified by the light stimulus which must accompany the setting up of rhizomes in apparatus and the carrying out of any visual observations. Infra-red photographic recording provides a safe and convenient experimental approach, but adequate time of recovery from the light stimulus given in setting up is essential.

(*b*) *The inversion or displacement effect*. The nature of the growth reactions brought about by inverting the rhizomes will be clear already from the data given in Figs. 5–10. After a short 'latent period' of about 30–40 min., rapid elongation of the side of the rhizome that had been the *lower* side during exposure to the stimulus occurs; so that, in a rhizome kept permanently inverted, the rhizome bends sharply upwards. This is followed by an equally sharp bending in the opposite direction, and after a number of bendings up and down of decreasing magnitude the rhizome finally becomes straight and continues to grow straight at the normal angle of 90°. Essentially the same type of reaction is evoked by the stimulus of rotating the rhizome through 90° about its long axis, which was considered on p. 185.

The simplest explanation is that something moving towards the lower surface during the stimulus causes an acceleration of growth in that region. If the 'something' is considered to be a hormone which initiates a growth process, then an explanation must be suggested to account for the straight growth before the movement of the hormone downwards. The symmetry of the rhizome and the fact that recovery from the stimulus brings about a condition apparently exactly similar to that existing before the stimulus, but with a different anatomical region as the physical lower side, seems to make necessary at least a two-hormone or two-particle hypothesis of the perception mechanism.

The hormone in its original position must be neutralized by an anti-hormone, or, alternatively, a second hormone capable of evoking growth acceleration, but located on the opposite side of the rhizome, could be postulated as the neutralizing agent. The rhizome after some 12 hours recovers from the stimulus and is in a condition exactly similar to that found before the stimulus, so that a change in position of both hormone and neutralizing agent must be postulated with the proviso that the movements occur at different rates.

These hypotheses are illustrated diagrammatically in Fig. 20. The initial distribution of hormones is shown in diagrammatic transverse sections of rhizomes at the extreme left. The other columns show, respectively, the condition immediately after the change of position, at the time when redistribution of hormones has commenced and at final equilibrium. The initial direction of bending is indicated by the arrows. The two upper rows of diagrams refer to the effects of inversion and the two lower to those of 90° rotation around the longitudinal axis.

The most simple form of hormone hypothesis of a geotropic reaction, seen, for example, in the Went-Cholodny hypothesis, is that the hormone is an essential metabolite displaced under the influence of gravity and that in the elongating tissue it is the concentration of this hormone which determines the rate of growth. In the hormone and antihormone hypothesis as illustrated the former has been supposed to have the greater mobility. The change in positions as shown in the third figure from the left should cause upward bending which should cease when the two substances have both attained their new equilibrium positions as shown in the right-hand diagrams.

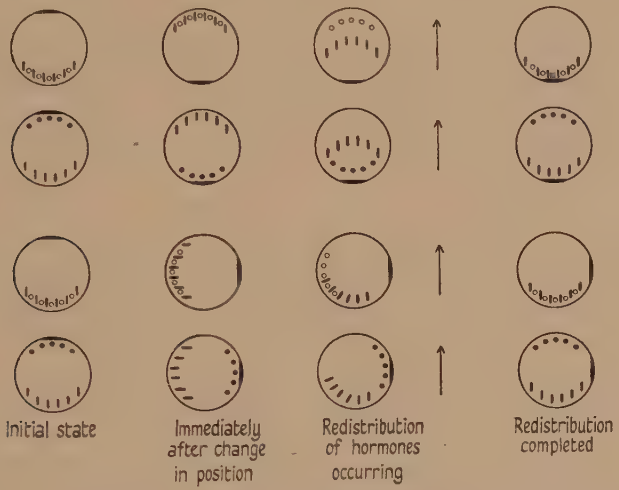
Initial state
Immediately after change in position
Redistribution of hormones occurring
Redistribution completed

FIG. 20. Diagrammatic transverse sections of rhizomes illustrating two types of hypothesis of the mechanism of perception of gravity. The two upper rows illustrate the effect of inversion and the two lower rows that of rotation of 90° around the long axis. The upper row of each set illustrates the hormone : antihormone hypothesis (fast-moving hormone shown as I I I and slow antihormone as ○ ○ ○). The lower row of each series illustrates the hypothesis involving two accelerating hormones (the fast-moving one being shown as I I I and the slow as ● ● ●). Original upper side of rhizome indicated by thickening of the circumference and the initial direction of curvature is indicated by the arrows.

This simple scheme cannot explain the whole of the displacement effect that we observe, because the response consists of first a relative acceleration of the lower side followed by retardation and a further series of accelerations and retardations which gradually die out. We have at present no evidence to frame hypotheses of the mechanism, but we could suggest that the accumulation of hormone as shown in the third column acts as the initiator at the lower side of the rhizome of a cyclical sequence of growth reactions starting with a relative acceleration.

The necessity of this sort of view of the perception mechanism is further emphasized by the results which have been observed in those experiments where rhizomes were inverted for short periods of 30 and 10 min. On return to the position which they had before the short period of inversion, any particles or molecules displaced during the stimulus must presumably travel back to their original positions, and in the case for example of the 10-min. stimulus one would imagine that in the 10 min. following the return to the original position the *status quo* would be restored. In the case of the 10-min. inversion, some 20 min. after the return of the rhizomes to their original position and consequently some 10 min. or so after the return of the displaced hormones or particles to their original positions, the rhizomes begin to respond just like the permanently inverted ones in that the side which had been *lowermost* during the stimulus grew the more rapidly at first. This was followed by the usual fluctuations in growth rates resulting in up-and-down bending movements which finally died out, the rhizome becoming straight and continuing to grow straight.

The response began after anything displaced by gravity had been returned to its original position by gravity acting in the opposite direction, so it seems clear that the receptive mechanism is simply the initiator of a series of complex changes, since the reaction continues for many hours although the displaced agents (particles or hormones) were only displaced for 10 min.

A mechanism like that postulated by Went and Cholodny and supported by Dolk (1929) is not applicable. This assumes that auxin accumulates on the lower side and the response, which is an accelerated growth of that side, is due to the presence of extra auxin at the time when the accelerated growth is occurring. The perception of the stimulus is considered to consist in the downward migration of auxin possibly associated with an electrical assymmetry of the horizontally lying organ.

(*c*) *Effects of displacement through 45° and 90°*. The experimental data have already been dealt with on pp. 189–91. One feature which is shown most clearly following the 90° displacements deserves full discussion. On turning rhizomes into the 180° position (pointing vertically up) or to 0° (vertically down) growth changes are initiated very like those caused by inversion or rotation. The side of the rhizome which had been uppermost immediately before the stimulus at first extends the more rapidly, so that the rhizome bends over markedly with the former lower side concave for about 3–4 hours; then, as with inversion stimuli, curvature in the opposite direction occurs, so that the rhizome rapidly straightens out and commonly bends farther so as to curve in the opposite direction. These reactions are so exactly similar to those following inversion that one is tempted to put forward the same sort of hypothesis of the reception mechanism. This is illustrated in Fig. 21.

The hormone-antihormone hypothesis only is represented. As a result of turning the rhizome into a vertical position the receptor hormone is supposed to tend towards a symmetrical distribution through the organ by diffusion, since its former status concentrated on the lower side is regarded as due to the transverse gravitational field. The presence of the hormone uninhibited by the antihormone on the former upper (i.e. left hand) side of the up-pointing rhizome initiates the acceleration of elongation of this side which is then followed by the further rhythmic growth changes. The down-pointing rhizomes behave similarly, where the former upper side is shown in the diagram as on the right.

In the case of the rhizomes which received the stimulus of inversion, they remained in the stable horizontal position, after these rhythmic growth changes due to the stimulus had died away, but with those displaced into the vertical position this is not the case. Rhizomes displaced to oblique angles turn after a number of hours towards the horizontal position which eventually is attained. Vertically placed rhizomes are in a metastable state, and slight displacement to one or other side might be expected to be followed by eventual turning in that direction to the final horizontal plane. Inspection of the data shown in Figs. 13 and 14 shows that this sort of behaviour is in fact realized experimentally. Placing the rhizome vertically initiates the displacement effect and the rhizome bends first to, say, the right; then the direction of movement is reversed. This reversed movement is opposed to the innate diageotropism of the rhizome (using the term diageotropism in accordance with the hypothesis which is discussed later and is indicated diagrammatically in Fig. 22 *b*), and in a great many cases it soon dies out and the rhizome resumes its original direction of movement towards the horizontal. In other cases the reversed movement carries the tip back beyond the metastable vertical position, and when this happens the diageotropism begins to work in the same sense and the movement continues in this direction towards the horizontal plane. Consequently, the final result of placing rhizomes vertical is that they turn into the horizontal plane in such a way that in some the former upper and in others the former lower side is uppermost.

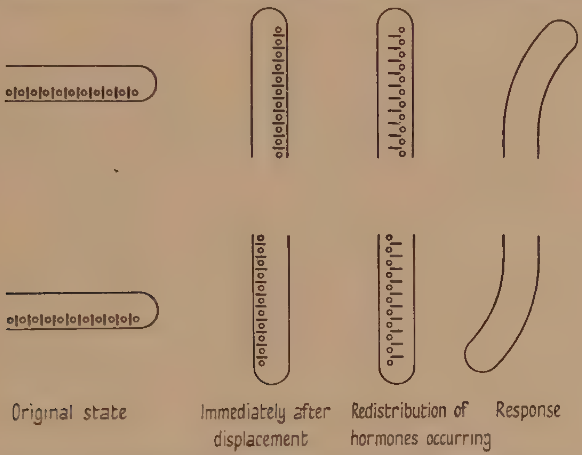
Original state
Immediately after displacement
Redistribution of hormones occurring
Response

FIG. 21. Diagrammatic representation of changes following displacement into the vertical up-and-down positions on the basis of the hormone : antihormone hypothesis. Conventions as in FIG. 20.

We see now that the mechanism of a further form of gravitational response must be considered: the turning from the oblique into the horizontal position which is most conveniently considered first in connexion with final behaviour of rhizomes displaced to the angles of 45° and 135° to the vertical down position. The actual experimental results described on p. 190 show that displacement from 90° to 45° (oblique down) produces a response similar to that evoked by displacement from 90° to 0°: the former upper side at first grows more quickly, causing the rhizome to turn down farther, and it then swings back as usual, and then continues to make a strong curvature towards the horizontal plane.

Displacement to the 135° position (oblique up) appears to evoke a similar reaction after the usual latent period. The upper side (before the stimulus) grows the more quickly so that the rhizome in the first phase of the displacement reaction moves strongly towards the horizontal, and this seems to prevent the back swing since a gradual return to the horizontal plane continues. The 'explanation' of the displacement stimulus formerly given is just as applicable here as in the former cases considered. Displacement to 225° (45° slope up) produces a characteristic very rapid bending towards the vertical which is the typical displacement effect due to the more rapid extension of the former upper side of the rhizome. This is followed by bending in the opposite direction and a gradual movement towards the horizontal. The behaviour conforms to that following other angular displacements.

We are therefore concerned with the diageotropic curvature which causes eventual turning into the 90° position. Irrespective of the actual chemical mechanism which must follow a displacement of particles or molecules in the gravitational field, the stimulus must be the change in the direction in which gravity acts on the organ. Comparing the incidence of gravity in the horizontal and oblique positions (see Fig. 22 *a*) we can regard the force of gravity as being resolvable into two components acting in the transverse and longitudinal directions (referred to in the German literature as *Querkraft* and *Längskraft* respectively). The transverse component acts in the same direction through the organ whether it slopes upwards or downwards. Unless the rhizome changes sensitivity, becoming positively geotropic in the up position and negatively geotropic in the down, a most improbable assumption, it seems clear that this component cannot be the operational stimulus.

On the other hand, the longitudinal component acts basipetally in the up-pointing rhizomes and acropetally in those pointing down, and is missing in those placed horizontally and might therefore act as the controlling stimulus. This suggestion is supported by the fact that a slight deflexion, α, from the horizontal affects the magnitude of the longitudinal component far more than the transverse, since the longitudinal component is $g \sin \alpha$ and the transverse is $g \cos \alpha$. An 8° deflexion from the horizontal, for example, decreases the transverse component 1 per cent. and raises the longitudinal component from zero to about 14 per cent. of its maximum; an 18° deflexion causes a decrease of 5 per cent. in the transverse component and raises the longitudinal component from zero to 31 per cent. of its maximum.

The suggested stimulatory activity of the longitudinal component might be looked on as due to its effect on the rate of growth.

A preliminary experiment in which short lengths (50 and 100 mm.) of rhizome were planted in soil in the normal or 90° position and also in the vertically up-and-down positions lent support to this view; it appeared that rhizomes in the vertically up, 180°, position grew considerably less rapidly than the remainder and the 0° placed rhizomes grew the most rapidly of the three series. Since aeration and water-supply conditions were not adequately equalized, the experiment was not well designed and detailed results are not given here.

Growth rates of rhizomes which were being examined by the infra-red recording technique, however, were easily obtained from the projected images on the screen which were normally examined in study of their angular displacements. The results of a number of typical experiments are collected together and summarized in Table II. It will be noted that a significant increase in growth rate is observed on turning the rhizomes from the horizontal to the oblique or vertical down positions and an even more strikingly significant decrease occurs when the corresponding change to the vertical or oblique up position is made.

TABLE II

Effect of position of rhizomes on rate of growth. In each case the mean growth rate during the 6 hours immediately before the change in position is compared with that in the succeeding 6 hours. Rhizomes which had a mean growth rate less than 3 mm./24 hours during experimental periods are not included. HS indicates significance beyond 0·01 level, S beyond 0·05 level

Experiment numbers	No. of rhizomes	Position	Growth rate mm. per 24 hr.	Difference and standard error	Difference as a percentage of rate in horizontal position	
7/49, 22/49 27/49	39	Horizontal	6·51	−2·29±0·448	−35·2±6·88	HS
		Vertically upwards	4·22			
6/49, 12/49	36	Horizontal	6·46	+0·91±0·384	+14·09±5·94	HS
		Vertically downwards	7·37			
2/49, 20/49	26	Horizontal	7·05	−1·28±0·423	−18·15±6·00	HS
		Obliquely upwards	5·77			
3/49, 21/49	35	Horizontal	6·91	+0·69±0·33	+10·0±4·77	S
		Obliquely downwards	7·60			

On a hormone hypothesis, the inference is that the longitudinal movement of a growth substance in the rhizome is affected by gravity. If we assume that auxin is produced at a constant rate in the tip and moves basipetally, then an increased rate of movement would decrease the concentration in the elongating region, since it would traverse this region more rapidly, and a decreased rate of movement would increase it.

One requires two further postulates in order to use these ideas and scanty facts as the basis of a theory of diageotropism. Firstly, that this growth promoter also migrates transversely in the tissue, accumulating on the lower sides of the rhizomes. Secondly, that in a horizontal rhizome normally at equilibrium, its activity on the lower side is balanced by an immobile or less mobile antisubstance. The consequences of displacement on the basis of this hypothesis are indicated diagrammatically in Fig. 22 *b*. The figure is self-explanatory, but one should add that this diatropic auxin most probably should not be equated with the receptive agents which were regarded as necessary to account for the inversion or displacement effects.

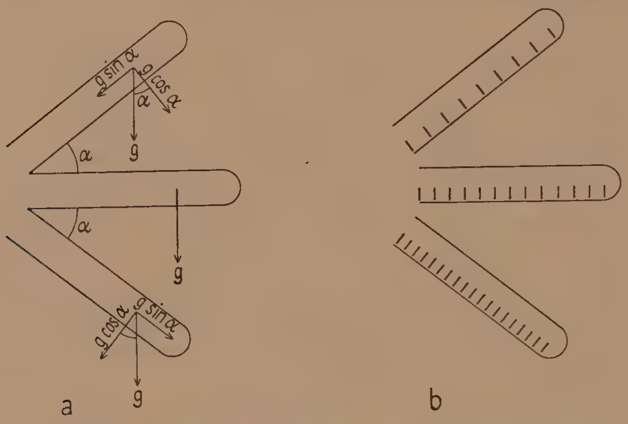

FIG. 22. Diagram *a* illustrates direction of the longitudinal and transverse components of gravity. Diagram *b* illustrates the suggested effect of this on the concentration of an accelerator hormone. The anti-hormone (not shown in diagram) is postulated as just balancing the accelerator in the horizontal position; it should thus be in excess in the up position and in deficit in the down position. For further details see text, p. 200.

(*d*) *Effects of carbon dioxide.* These effects have already been illustrated in the experimental section, and it remains to point out that these and other results are consistent with the view that the normal fairly accurate horizontal growth in darkness is due to the balance in activity of at least two substances. This balance, it would seem, is disturbed by light and also by the ventilating of the rhizomes with air containing 5 or 10 per cent. of carbon dioxide. Since in the presence of carbon dioxide the rhizomes finally turn slowly upwards, one could suggest the possibility that a process of carbon-dioxide fixation increases the supply of the diatropic auxin and displaces the whole system from its normal balance. We have, however, no data on which to advance such speculations, and indeed it seems clear that the next stage of the study must be the attempt, already commenced in this laboratory, to effect separations and quantitative estimations of a range of growth-promoting and retarding substances extractable from plant tissues. This is an essential forerunner of any attempts at isolation and eventual chemical characterization. One may dismiss, we feel, as inadequate any 'one auxin' hypothesis of geotropic behaviour.

(*e*) *Klinostat experiments.* The idea that one could separate an orthogeotropic component of plagiotropic response from other factors such as epinasty or inherent diatropism suggested repetition of klinostat experiments. It is now realized that the occurrence of the displacement effect, following either inversion or rotation about the longitudinal axis, is bound to make interpretation of rhizome behaviour on a klinostat difficult. It seemed unlikely that klinostat behaviour would assist in further analysis of plagiotropism in material such as *Aegopodium* which shows such pronounced displacement reactions.

(*f*) *Rhizome biology.* A final paragraph, in which one is liable to enter the dangerous field of teleology, may not be amiss. The complex range of response which has been described has been relatively fully investigated in the special case of *Aegopodium* and preliminary work shows that *Circaea* is probably closely similar. Certain other rhizomes (see p. 193) have a geotropic mechanism which is apparently not affected by light; we are not yet certain whether they are negatively phototropic. It is certainly clear that the mechanisms worked out in detail in *Aegopodium* bring the rhizomes back into the correct horizontal plane if they happen to get deflected by hitting against stones or obstacles in the soil. If, in pursuing this horizontal course, they grow out to the surface from a sloping bank, the light effect, a tendency to grow downwards, corrects this and keeps them below ground. In the same way, if their horizontal course takes them down too deep by burrowing into a bank, the rising carbon-dioxide concentration puts the diatropic mechanism out of balance and the rhizome apex starts coming up until it reaches a zone with a suitable carbon-dioxide concentration where it will start levelling off. Incidentally, one may note that our source of *Aegopodium* originated in a garden consisting of light sandy Surrey soil where the rhizomes tended to be growing 3–7 cm. below the surface when other vegetation was cleared away. Some of this material was grown for experimental purposes in dark humus rich soil in which the level of the rhizomes was rarely much greater than 4 cm. It seems very likely that an important controlling factor is the concentration of carbon dioxide in the soil air.

(*g*) *Comparison with other plagiogeotropic behaviour.* The angle of inclination to the vertical taken up by individual *Aegopodium* rhizomes can be anything from about 65° to 120°, depending on radiation and carbon-dioxide concentration in the air, so that the normal nearly horizontal position may be regarded as largely fortuitous. This diatropism is in fact a special case of plagiotropic behaviour obtaining in the dark with low carbon-dioxide concentrations.

One may note meantime that the behaviour of the lateral branches of *Asparagus plumosus*, which was very carefully investigated by Rawitscher (1930), appears to agree well with the picture which we are presenting. A small angular displacement causes the laterals to bend back into their usual horizontal position, but on displacement through 90° they turn in such a way that the former upper side becomes convex. This happens also when the shoot is turned so that the apex points obliquely downwards; laterals in this case which are on the upper side of the shoot at first turn up, becoming nearly vertical, and then bend in the opposite direction, becoming horizontal. This is essentially similar to the behaviour of *Aegopodium* rhizomes and may well be brought about by a similar mechanism.

It seems possible that other cases of plagio- and diatropic behaviour could be 'explained' by mechanisms similar to that which we provisionally suggest. In the organ when at its equilibrium inclination the postulated accelerator and the antisubstance balance each other; rise in inclination by reducing the accelerator concentration would initiate downward movement, and vice versa. On this hypothesis the factor determining what equilibrium inclination is found in any given organ is the ratio between the concentration of a more mobile accelerator and a less mobile antisubstance; relatively large values of this ratio would be expected to result in high inclination angles and vice versa.

Experimental testing of this point of view can best be effected by separation and assay of growth substances, and procedures for carrying out these operations are being developed in this laboratory.

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Microbiological Synthesis of Fat

PRELIMINARY SURVEY OF THE FAT-PRODUCING MOULDS

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SUMMARY

1. A survey of potential fat-producing moulds has shown that at least 40 strains from 10 species are of interest.

2. These have been grown on five different media and *A. nidulans*, *P. spinulosum*, *P. javanicum*, *P. piscarium*, *P. flavo-cinereum*, *P. oxalicum*, *A. flavus*, *A. flavipes* have shown the most promise.

3. The fat content on felt weight was maximal at 39·7 per cent. with *A. flavipes* and 34·6 per cent. with *F. lini*, and on used sugar was maximal at 9·3 per cent. with *A. flavipes* and 6·7 per cent. with *A. nidulans*.

4. The potential value of micro-organisms as fat producers is discussed with reference to the moulds.

A WORLD shortage of food, arising from the sustained rate of increase in population and the continued demand for a higher standard of living, has been anticipated and emphasized recently by such authorities as Russell (1949) and in the speeches and writings of Boyd Orr. The synthetic efficiency of micro-organisms has, therefore, naturally attracted attention, and Woodward (1950), for example, has suggested that the highly efficient capabilities of the algae might provide 'creatable resources' by their initial production of basic carbohydrate material. This material by suitable hydrolytic treatment would then yield the substrates for the formation of the final products by other micro-organisms.

In the search for fat-producing moulds the general background has been reviewed by Bernhauer (1943), Kleinzeller (1948), Foster (1949), Hesse (1949), and Raveux (1949). From our survey (Gregory, 1950; Woodbine, 1951) some 40 strains from 10 species appeared suitable for study and they are shown in Table I.

EXPERIMENTAL

Strains. All the strains were obtained from the Baarn Culture Collection with the exception of the strains of *Mucor circinelloides*, obtained through the courtesy of Professor K. Bernhauer, *Fusarium lini* Bolley and *F. lycopersici* by courtesy of Professor F. F. Nord, the *Mortierella* sp. by courtesy of Mr. L. D. Galloway, and *Penicillium spinulosum*, which was isolated as a casual contaminant from a *M. albo-ater* culture (see Table IV).

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Journ. of Experimental Botany, Vol. II, No. 5.

TABLE I

Mould species and strains examined for fat production

Aspergilli

A. clavatus Desm.
A. flavipes (Bain. et Sart.) Thom et Church
A. flavus Link
A. insuetus Bainer
A. minutus Abbott
A. nidulans Eidam
A. niger v. Tieghem

Penicillia

P. aurantio-brunneum Dierckx
P. flavo-cinereum Biourge
P. funiculosum Thom
P. javanicum v. Beyma
P. luteum Zukal
P. oxalicum Currie et Thom
P. piscarium Westling
P. purpurogenum v. *rubri-sclerotium* Thom
P. roqueforti Thom
P. Soppi Zaleski
P. spinulosum

Fusaria

F. bulbigenum (1) Cke. et Mass. var. *tracheiphilum* (E. Sm.) Wr.
F. bulbigenum (2) Cke. et Mass. var. *blasticola* (Rostr.) Wr.
F. bulbigenum (3) Cke. et Mass.
F. graminearum
F. lini Bolley (1)
F. lini Bolley (ex Nord) (2)
F. lycopersici (ex Nord)
F. oxysporum (Schl.)

Mucorales

M. albo-ater Naoumoff
M. circinelloides v. Tieghem (1)
M. circinelloides M 69 (ex Bernhauer) (2)
M. mucedo Bref
M. plumbeus Bon.
M. Ramannianus Moller
M. spinosus v. Tieghem

Miscellaneous

Cladosporium herbarum Link
Mortierella sp. (ex Galloway)
Oospora lactis (Fres.) Sacc.
Paecilomyces varioti Bainer
Rhizopus nigricans Ehrenberg.
Rhizopus oryzae Went et Pr. Geerl.
Trichoderma viride Pers. ex Fr. (1) (*T. lignorum* (Tode) Harz).
Trichoderma viride Pers. ex Fr. (2) (*T. Koningi* Oud.)
Ustilago zeae (Beckm.) Unger.
Zygorhynchus Moelleri Vuill.

TABLE II

Composition of culture media, all quantities as g. per l.

	Medium A (Prill *et al.*, 1935)	Medium B (Lockwood *et al.*, 1934; Ward *et al.*, 1935)	Medium B2	Medium C (modified Czapek-Dox of Raistrick and Vincent, 1948)	Medium D (Bernhauer *et al.*, 1948)
Ammonium nitrate .	10·0	2·25	2·25	—	—
Ammonium sulphate .	—	—	—	—	0·10
Calcium carbonate .	15·0	—	—	—	—
Corn steep liquor (C.S.L.) . .	—	—	5·0 (ml.)	—	—
Ferric chloride . .	0·16	—	—	—	—
Ferrous sulphate .	—	—	—	0·10	—
Glucose	200·0	225·0	225·0	50·0	10·0
Magnesium sulphate .	5·0	0·25	0·25	0·50	0·25
Potassium chloride .	—	—	—	0·50	—
Potassium dihydrogen phosphate . .	6·8	0·30	0·30	1·0	0·50
Sodium nitrate . .	—	—	—	2·0	—
Zinc sulphate . .	0·05	—	—	—	—
pH	7·0	5·1	5·7	5·9	5·1

Maintenance. The strains were kept on malt wort containing 2 per cent. agar, prepared according to the method of Tosic and Walker (1944), and were subcultured at intervals of 3 months.

Media. Five media, of the composition shown in Table II, have been used for investigating the potentialities of the strains selected. All the chemicals used were B.D.H. 'Analar' grade, except for the glucose, which was the B.P. ('Dextrosol') grade of Corn Products Ltd. All media were made up with distilled water. The pH was determined by Johnson's papers prior to autoclaving for 15 minutes at 10 lb. pressure.

Harvesting. The moulds were grown in quintuplicate using 100-ml. Erlenmeyer conical flasks each containing 25 ml. medium and were sterilized by steaming for 30 minutes. The pH was determined by using Johnson's papers. The residual medium volume was obtained and the felts were transferred to filter-papers. After 2–3 washings with water on the Buchner and air-drying for 2–3 days, the felts were weighed and extracted.

Estimation of fat. The air-dried felts were dried in the water-oven, at not more than 60° C., allowed to cool, weighed, and ground, using Ballotini (Grade 12, Chance Bros.) as a dispersing agent. The relatively homogeneous product from five flasks was transferred to Whatman ether-extracted thimbles and 'hot extracted', in Quickfit and Quartz extraction units, for 18 hours using light petroleum (40–60° C.). The light petroleum was removed on the steam-bath and the flasks were dried at 60° C. for 30 minutes, cooled and weighed. This weight is referred to as weight of fat.

TABLE III

Moulds giving the highest yield of felt and fat when grown on medium A

Mean felt weight (g.)		Fat (g./100 g. dry felt)		Fat (g. 100 g. sugar utilized)	
A. nidulans	2·13 (±0·80)	*U. zeae*	(36·6)	*P. javanicum*	3·5
A. flavipes	2·09 (±0·22)	*P. javanicum*	7·9	*A. nidulans*	2·4
P. piscarium	1·89 (±0·05)	*Z. Moelleri*	7·7	*P. piscarium*	2·0
A. clavatus	1·55 (±0·20)	*A. nidulans*	7·6	*P. spinulosum*	1·8
P. javanicum	1·44 (±0·03)	*P. spinulosum*	7·2	*P. flavo-cinereum*	1·1
P. aurantio-brunneum	1·44 (±0·02)	*F. bulbigenum* (2)	6·9	*P. roqueforti*	0·90
A. flavus	1·39 (±0·20)	*M. albo-ater* (25° C.)	(6·5)	*P. aurantio-brunneum*	0·79
P. flavo-cinereum	1·38 (±0·02)	*Rh. nigricans* *F. lini* Bolley (1) (25° C.)	5·9	*A. flavipes*	0·78
P. Soppi	1·36 (±0·16)	*T. viride* (1)	5·4		
P. spinulosum	1·19 (±0·15)	*M. plumbeus*	5·3	*Rh. nigricans*	0·77
P. purpurogenum	1·18 (±0·016)	*P. flavo-cinereum*		*P. oxalicum*	0·72

Table IV

Moulds giving the highest yield of felt and fat when grown on medium B

Mean felt weight (g.)		Fat (g./100 g. dry felt)		Fat (g./100 g. sugar utilized)	
A. flavus	1·18	*M. albo-ater* (contaminated with *P. spinulosum*)	12·4	*P. flavo-cinereum*	4·74
C. herbarum	0·43 (±0·07)	*Rh. nigricans*	8·3	*A. flavus*	2·40
P. flavo-cinereum	0·43 (±0·25)	*P. flavo-cinereum*	7·6	*M. albo-ater* (contaminated with *P. spinulosum*)	2·37
P. javanicum	0·39 (±0·12)	*A. flavus*	5·7	*P. javanicum*	2·04
M. albo-ater (contaminated with *P. spinulosum*)	0·43 (±0·04)	*Rh. oryzae*	4·9	*Rh. oryzae*	0·84
P. purpurogenum	0·41 (±0·20)	*P. javanicum*	3·5	*C. herbarum*	0·45
A. niger	0·50	*P. purpurogenum*	1·3	*P. purpurogenum*	0·35
P. varioti	0·21 (±0·05)	*A. niger*	0·86	*Rh. nigricans*	0·35
Rh. oryzae	0·077 (±0·003)	*P. varioti*	0·76	*A. niger*	0·33
Rh. nigricans	0·043 (±0·010)	*C. herbarum*	0·70	*P. varioti*	0·16

Table V

Moulds giving the highest yield of felt and fat when grown on medium B2

Mean felt weight (g.)		Fat (g./100 g. dry felt)		Fat (g./100 g. sugar utilized)	
P. javanicum	1·80 (±0·14)	*F. lini* (25° C.) (1)	34·6	*A. nidulans*	6·3
A. nidulans	1·27 (±0·12)	*A. clavatus*	20·7	*F. lini* (25° C.) (1)	5·6
P. oxalicum	1·25 (±0·10)	*A. nidulans*	20·2	*P. spinulosum*	4·4
C. herbarum	1·19 (±0·23)	*P. spinulosum*	19·0	*P. flavo-cinereum*	4·1
P. flavo-cinereum	1·10 (±0·17)	*P. oxalicum*	15·7	*P. oxalicum*	4·0(7)
P. purpurogenum	1·09 (±0·09)	*P. flavo-cinereum*	15·2	*A. insuetus*	3·3
P. spinulosum	1·08 (±0·08)	*Z. Moelleri*	14·0	*P. javanicum*	3·2
A. niger	1·03 (±0·17)	*Rh. nigricans*	13·4	*A. clavatus*	3·0
P. aurantio-brunneum	0·95 (±0·04)	*F. graminearum* (25° C.)	12·6	*A. flavus*	1·8
A. insuetus	0·93 (±0·32)	*Rh. oryzae*	11·0	*P. aurantio-brunneum*	1·4

Residual glucose. The liquid contents of the series of flasks were collected and made up to a known volume, usually twice that of the original volume. If the estimation was not proceeded with immediately a portion was sterilized under free steam for 15 minutes. The glucose was estimated according to the method of Willstätter and Schüdel (1918).

Shaker. The shaker used with medium D was of 50-flask capacity, placed in 2 trays, and operating at 66 r.p.m., with a 2½-in. throw.

RESULTS

The detailed results for all the media are not given. In order to conserve space only those for the best of each series are presented. Those figures in brackets may not be as accurate as the remainder since the actual weight of felt or fat was rather small. The results from medium A after 13 days' growth are shown in Table III, in which the best 10 moulds are presented in decreasing order according to mean felt weight, g. fat per 100 g. dry felt or g. fat per 100 g. sugar utilized. The standard deviations are given immediately underneath the mean felt weight figures. All the cultures were grown at 30° C. unless 25° C. is specified.

TABLE VI

Moulds giving the highest yield of felt and fat when grown on medium C

Mean felt weight (g.)		Fat (g. 100 g. dry felt)		Fat (g. 100 g. sugar utilized)	
P. javanicum	0·35 (±0·01)	*A. flavipes*	39·7	*A. flavipes*	9·3
P. Soppi	0·31 (±0·03)	*Mortierella* sp. (21 days at 25° C.)	34·9	*A. nidulans*	6·7
A. flavipes	0·27 (±0·06)	*U. zeae*	30·2	*P. oxalicum*	5·7
P. spinulosum	0·27 (±0·01)	*F. lini* (1)	28·6	*U. zeae*	4·7
P. piscarium	0·26 (±0·02)	*A. nidulans*	26·4	*Mortierella* sp.	3·9
A. nidulans	0·24 (±0·02)	*P. oxalicum*	23·2	*A. insuetus*	2·6
C. herbarum	0·19 (±0·03)	*A. insuetus*	16·6	*P. spinulosum*	2·6
A. niger	0·19 (±0·04)	*M. circinelloides* (v. Tieghem) (1)	15·7	*P. javanicum*	2·5
P. varioti	0·17 (±0·01)	*A. clavatus*	14·4	*P. flavo-cinereum*	2·3
P. funiculosum	0·17 (±0·06)	*M. plumbeus*	14·2	*P. Soppi*	2·0

The results on medium B, after 14 days' growth at 30° C., are presented in Table IV in a similar manner to those of medium A. These were somewhat disappointing compared with the results with medium A. When modified, however, by the addition of 0·5 per cent. Corn Steep Liquor (medium B 2), the overall results were much improved. The results for medium B 2, after growing for 11 days at 25 or 30° C., are shown in Table V.

The results on the modified Czapek-Dox medium of Raistrick and Vincent (1948), medium C, after 14 days' growth at 30° C., are depicted in Table VI.

The results with the shake cultures are shown in Table VII. Owing to the capacity of the shaker being limited to 10 moulds, it was necessary to proceed in stages. Ten flasks of each mould were prepared and inoculated; 5 were shaken cultures and 5 were stationary cultures. These experiments had to be carried out in the laboratory and the temperature varied from 12·5° C. at night to 19·5° C. during the day. The cultures were grown for 14 days, with shaking during the day only.

TABLE VII

Moulds giving the highest yield of felt and fat when grown on medium D

[illegible]			[illegible]			[illegible]		
P. [illegible]		0·061 (±0·0005)	*M. ramannianus*		55·5	*A. clavatus*		4·6
	shaken	0·038 (±0·0002)	„	shaken	16·7	„	shaken	3·7
P. [illegible]		0·049 ([illegible])	*M. spinosus*		46·7	*A. minutus*		4·4
	shaken	0·047 (±0·0017)	„	shaken	28·4	„	shaken	1·0
P. [illegible]		0·063 ([illegible])	*M. circinelloides v. Tieg.* (5)		42·7	*A. flavus*		3·3
	shaken	0·086 ([illegible])	„	shaken	45·4	„	shaken	5·7
A. [illegible]		0·049 ([illegible])	*M. albo-ater*		41·8	*M. circinelloides v. Tieg. (5)*		3·8
	shaken	[illegible] (±0·0052)		shaken	[illegible]		shaken	[illegible]
M. [illegible]		0·029 ([illegible])	*Rh. oryzae*		35·8	*M. albo-ater*		3·9
	shaken	[illegible] ([illegible])		shaken	[illegible]		shaken	[illegible]
A. [illegible]		0·026 (±0·0021)	*A. minutus*		34·9	*M. spinosus*		3·3
	shaken	[illegible] (±0·0024)		shaken	[illegible]		shaken	[illegible]
P. [illegible]		0·027 ([illegible])	*P. oxysporum*		33·9	*F. oxysporum*		2·8
	shaken	[illegible] ([illegible])		shaken	[illegible]		shaken	[illegible]
P. [illegible] carum		0·027 ([illegible])	*M. mucedo*		33·3	*P. purpurogenum*		2·7
„	shaken	0·027 ([illegible])	„	shaken	14·6	„	shaken	0·9
P. [illegible]		[illegible] ([illegible])	*P. [illegible]*		[illegible]	[illegible]		[illegible]
	shaken	[illegible] ([illegible])		shaken	[illegible]		shaken	[illegible]
P. aurantio-brunneum		0·024 (±0·0003)	*A. flavus*		28·1	*Rh. oryzae*		2·5
„	shaken	0·024 ([illegible])	„	shaken	35·5	„	shaken	1·5

DISCUSSION

Although the concept of using micro-organisms as primary food producers in practice is of fairly recent origin, they have been used in an accessory capacity for many years. Algae have provided thickeners for jams and soups in the form of alginates or agar, the use of yeasts in the baking and brewing industries is almost immemorial, and the moulds have provided colour and flavour in, for example, some of the more renowned cheeses.

The algae *Chlorella pyrenoidosa*, *Tribonema aequale*, and *Pennales* have recently been shown (Milner, 1948; Spoehr and Milner, 1947–8, 1949; Harder and Witsch, 1942, 1943; Fogg and Collyer, 1950) to be capable of producing fat (up to about 80 per cent.) or protein (up to about 75 per cent.), depending upon the experimental conditions, and the results with the pilot scale work at Stanford (Anon., 1950) can be anticipated with interest. The fat-yeast *Rhodotorula gracilis* (Enebo *et al.*, 1944, 1946; Lundin, 1950; Bunker and Macauley, 1950), with a content of over 50 per cent. fat on small-plant scale has superseded the yeasts *Endomyces vernalis* and *Oospora lactis* as a potential large-scale fat-provider.

The best of the moulds in the past, *P. javanicum* van Beyma (Lockwood *et al.*, 1934), and one of the top three of the present work, does not appear so conspicuous. The apparently lower potentiality of the moulds, however, may be partially mitigated by their ability to utilize industrial waste products and in the elaboration of potentially valuable material concomitantly in the liquor. (In this connexion the elegant production of vitamin B_{12} from spent streptomycin liquors is not without interest.) The overall production of fat from micro-organisms is limited by a maximum felt production of 50 per cent., as discussed by Brahmer (1947), and by the maximum conversion of carbohydrates to fat of 15 per cent. adduced by Rippel (1940) but modified to 18 per cent. by the results of Enebo *et al.* (1944, 1946), Pan *et al.* (1949 *a*, *b*), and Agarwal and Peterson (1949). This low efficiency of conversion renders the use of first-class carbohydrates prohibitive. Owing to cost of transportation it would be uneconomic to manufacture elsewhere than at or near the source of raw material supply (wood or paper manufacturing areas, sugar-producing centres) or to transfer the final products far to the site of their utilization. One of the major difficulties with food yeast has been that the palatability of the final product has been adversely criticized, although satisfactory organoleptic assessments may not have been carried out.

Although the content of protein in yeasts gives them a potentially higher overall food value, the best results obtained with the moulds (10 per cent. fat on used sugar; Woodbine, Gregory, and Walker, 1951) and the good initial results in shake and aerated cultures, obtained, for example, by Bernhauer *et al.* (1948), indicate that their potential value as food and fat-producers may be equally good.

ACKNOWLEDGEMENTS

The work described in this paper was initiated at the suggestion of the Committee on Chemical Microbiology of the Medical Research Council. One of us (M. W.) has been seconded to the Medical Research Council by the Scientific Civil Service (Ministry of Food) to enable him to participate in the work.

We should also like to express our appreciation to Professor F. F. Nord and Mr. L. D. Galloway for culture specimens and their interest in this work, and to Professor K. Bernhauer for the gift of a culture of *Mucor circinelloides*.

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Observations on the Gaseous Exchanges which take place between *Menyanthes trifoliata* L. and its Environment

I. THE COMPOSITION OF THE INTERNAL GAS OF THE PLANT

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SUMMARY

1. As part of a general investigation into the exchange of gases between the bogbean, *Menyanthes trifoliata*, and its environment, determinations have been made of the composition of the internal atmosphere of the plant at five different levels, viz. leafy shoot, three successive portions of stem, and roots.

2. Procedure is described for the analysis of small gas samples using a modification of the Bonnier and Mangin apparatus.

3. In darkness with the lower part of the plant surrounded by an atmosphere of nitrogen, it has not been possible to show the presence of a regular downwards gradient of oxygen, such as would be expected on a basis of normal gaseous diffusion.

4. Under these *Air Top / Nitrogen Bottom* conditions the roots are shown to contain between 12·5 and 17·5 per cent. of oxygen in their intercellular gas system. The portion of stem immediately adjacent to the roots showed an oxygen level of from 14·0 to 18·0 per cent.

5. In plants kept under *Nitrogen Top / Air Bottom* conditions in the dark for 48 hours, the concentration of oxygen in the roots fell to a level of 3·1 per cent. or below in three plants and to 6·0 per cent. in a fourth. The oxygen concentration in the adjacent lowermost part of the stem was as high as 18·0 per cent., depending on the degree of aeration of the solution bathing the stem. It is suggested that this distribution of oxygen can be related to the relative impermeability of roots and of stem endodermis to gaseous diffusion from without, and it is thought that the major part of the oxygen supply to the roots is transported to them through the stelar air passages.

6. Some evidence is presented to show that oxygen passes from the lower parts of the plant into the medium surrounding it.

INTRODUCTION

THE metabolism of aquatic and subaquatic plants in relation to the availability of their oxygen supply offers some points of considerable interest. For example, the root systems of such plants are most often found in a nearly oxygen-free environment, so that they are dependent for oxygen upon the gaseous exchanges which take place between the upper parts of the plant and the atmosphere. Conway (1940) has reviewed such work on gaseous exchange as has been carried out, and apart from the work of Laing (1940) and van Raalte (1940, 1943), there appears to have been little work on this problem over the last decade.

In *Cladium mariscus* it was established by Conway (1937) that oxygen occurred in the roots at nearly atmospheric concentrations and appeared to reach the roots by diffusion along a well-developed air-space system. Whereas the oxygen seems in *Cladium* to enter the plant mostly by way of the tufted leaf bases of the mature and even the withered leaves, this means of entry seems unlikely in non-caespitose plants. Depending upon whether the leaves are submerged or emergent, oxygen will presumably enter largely by way of the stomata of lamina or petiole, or more slowly by aqueous diffusion across the epidermis. Fairly high concentrations of oxygen in the root will depend on a low resistance to diffusion in the intervening tissues, and upon an adequate supply of oxygen capable of acting as a 'head' in the upper regions of the plant. The 'head' of oxygen will be higher during exposure to light; and on the assumption that a simple diffusion gradient exists, the following relation should hold:

$$\underset{\substack{\text{illuminated}\\ \text{leaves}}}{[O_2]} > \underset{\substack{\text{darkened}\\ \text{leaves}}}{[O_2]} > \underset{\substack{\text{upper}\\ \text{stem}}}{[O_2]} > \underset{\substack{\text{lower}\\ \text{stem}}}{[O_2]} > \underset{\text{roots}}{[O_2]}$$

Laing's work on *Nuphar advenum* (1940 *a*, *b*) supported this relation, and showed how illumination of the leaves may steepen the existing diffusion gradient in oxygen. On the other hand, change from darkness to light made very little difference to the oxygen gradient in other plants that he used, such as *Pontederia cordata*, *Typha latifolia*, and *Scirpus validus*. Conway (1937) pointed out that, in *Cladium mariscus*, leaves of plants after being held in darkness for a fortnight may contain 18–20 per cent. of oxygen, and for shorter periods of darkness this is our experience with bogbean. It is plain that even where a simple diffusion gradient obtains, it is not in all cases much modified by the incidence of photosynthesis.

It is proposed in the present paper to give an account of some work carried out with the bogbean, *Menyanthes trifoliata*. In this plant a small crown of leaves (seldom more than four or five in number) is borne terminally, whilst the adventitious roots present are mostly restricted to the lower half of the stem. Under normal growing conditions the water-level reaches to approximately the middle of the leaf petioles. The green rootstock is leafless and generally almost completely submerged, often growing horizontally along the top of the mud, within which the adventitious roots are buried. For convenience in the account which follows, the leafy apex will be designated as 'shoot' or 'leafy shoot', in contradistinction to the 'stem' or rootstock below it.

Certain preliminary work was carried out in which plants of *Menyanthes* were sealed into an apparatus in such a way that passage of gases between an upper and a lower gas chamber could only take place through the stem of the plant. The results seemed to indicate fairly clearly that, in the dark, oxygen could pass down through the plant by way of leaves exposed to a normal atmosphere in the upper chamber, and could be passed out in limited quantities into the lower chamber, which was nitrogen filled. Reversal of these conditions showed that if the stem was exposed to air whilst the leafy shoot was surrounded by nitrogen, oxygen passed in the reverse direction, and small amounts appeared in the nitrogen-filled upper chamber. These and other experiments will be dealt with in a subsequent paper, but together they led to the decision to attempt an analysis of the internal gases of the plant with a view to discovering under a variety of conditions what, if any, gaseous diffusion gradients existed between shoot and root which might account for the movement of oxygen.

EXPERIMENTAL METHODS

a. Treatment of experimental plants

For the purposes of this investigation plants were subjected to three sets of experimental conditions, which may be referred to briefly as: *Air Top/Nitrogen Bottom*, *Nitrogen Top/Air Bottom*, and *Air Top/Air Bottom*. For each experiment of the *Air Top/Nitrogen Bottom* series the stem of a plant was sealed into a 1-litre organic reaction flask, together with about 600 ml. of boiled distilled water. Nitrogen was bubbled through the flask for 1 hour, and it was then placed in a temperature-controlled dark room at 20° C. for 48 hours. At the end of this time the plant was removed and its internal gases were extracted and analysed. In the *Nitrogen Top/Air Bottom* series the leafy shoot of a plant was sealed into an inverted 500-ml. conical flask, so fitted with tubes that a stream of nitrogen could be blown through as before. The stem of the plant was immersed in water under varying conditions of aeration which are described subsequently. In the *Air Top/Air Bottom* series the stems were placed vertically in a jar of tap-water through which air bubbled continuously. As in the other series, the plants were left for 48 hours in the dark room at a temperature of 20° C.

b. The extraction of internal gases

Samples of the gases from the air-space system in stem and root of bogbean were withdrawn by means of a vacuum-extraction apparatus, which was a modification of that designed and used by Burton and Spragg (1948). That part of the plant selected for extraction, having been cut from the plant under water, was quickly transferred to the mercury trough, submerged, and then well agitated underneath the mercury surface to dislodge any superficially adhering air-bubbles. It was then introduced into the extraction tube. Ten seconds sufficed to extract enough gas for analysis from the given portion of the plant, and the extracted gas was transferred by manipulation of the reservoir to a sampling tube of the type used by Thoday (1913) and was stored over mercury.

For the purpose of analysis the plant was divided as follows into five sections, viz. the leafy shoot including the two short internodes below the lowest functional leaf; the upper, middle, and lower thirds of the stem; and the main roots. These are referred to in the histogram (Fig. 2) as S, I, II, III, and *R*, respectively. Fibrous roots were for the most part ignored. It was found possible to extract adequate samples of gas from all five portions in something under 30 minutes. As a routine measure, gas was always extracted from the roots first, and from the lowest portion of the stem next. Remaining portions of the plant awaiting extraction, immediately after cutting had their ends liberally smeared with a cold soft luting wax of beeswax and vaseline, and were kept immersed in cold water. Whereas diffusion and respiration may possibly have affected to a slight extent the gas composition of those portions of the plant extracted last, it is thought reasonable to assume that the gas content of root and lower stem could not have been affected significantly by these phenomena, and that furthermore the short time schedule would make it unlikely that wound reactions could have affected respirational activity in this period. Laing (1940*b*) drew attention to an initial rise in the output of carbon dioxide following the cutting or handling of his *Nuphar* rhizomes, although he took no account at all of this feature in his gas determinations, which he made upon internal atmospheres extracted from plugs of tissue removed from the rhizome by means of a cork borer.

c. The analysis of internal gases

1. *Apparatus.* The analysis of internal atmospheres for oxygen, carbon dioxide, and nitrogen content was carried out in a modification of Thoday's version (1913) of the Bonnier and Mangin apparatus (Fig. 1). The tube C, in which the gas sample was measured and in which absorption of carbon dioxide and oxygen took place, was a 30-cm. length of Pyrex capillary tubing, of uniform bore and about 0·5 sq. mm. in cross-sectional area. At B the tube was prolonged into an S-bend as shown; at the opposite end it was bent downwards through 90°, fused to a length of 7-mm. bore tubing bent again through 90°, and inserted into a piece of pressure tubing 6 in. long, with an internal bore of half an inch; this was gripped between a screw-controlled pressure plate and the baseboard, as shown at P. A tap T connected the pump to a small mercury reservoir. With the tap open, the whole apparatus could be

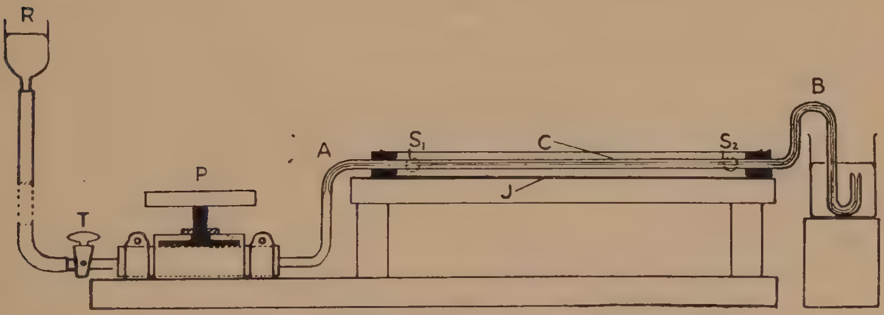

FIG. 1. Diagram of apparatus for gas analysis. For details see text.

cleaned through with the minimum of trouble. With the tap closed, manipulation of the screw-controlled mercury pump drew gases into or led them from the capillary tube. Temperature was controlled by use of a calibrated water-jacket J, through which water was passed by means of side-tubes, S_1 and S_2.

The hydrostatic pressure inside the capillary tube was kept constant during measurements by immersing the end of the tube in a beaker of mercury maintained at a constant level, which was approximately the same as that of the mercury in the horizontal part of the capillary.

2. *Procedure.* After thorough treatment with chromic cleaning solution the apparatus was washed through three or four times with tap-water, once with distilled water, and then finally with 5 per cent. sulphuric acid. Mercury was introduced into the reservoir and allowed to flow into the pump, care being taken to ensure that no air-bubbles had become trapped at the junction of the glass tubing and the rubber. As a further precaution, the end of the S-bend was connected to a suction pump, and any air that remained was dispelled. By means of the handscrew the whole of the capillary tube was filled with mercury, and the tip of the tube was then immersed, as shown, in a beaker of mercury. The sample of air was introduced into the capillary tube, and was generally about 100μl. in volume, corresponding to about 20 cm. of tube length. Measurement of the introduced bubble was made after carefully sweeping any fluid adhering to the walls of the capillary tube to that end of the bubble nearest (A), leaving a clean mercury meniscus at the end of the bubble nearest (B). The use of 5 per cent. sulphuric acid during the last wash, as suggested by Thoday (1913), facilitated the movement of the mercury thread, which in plain water tended to become more jerky in movement through adhesion to the walls of the capillary. The minimum reading obtained after a succession of such sweeps was taken as the final reading.

After measurement, all but about 5 cm. of the mercury seal was rejected, and the beaker of mercury was replaced by one containing 10 per cent. caustic potash, made up in well-aerated water. The potash was drawn in to wet the whole length of the capillary tube (thus neutralizing the traces of dilute sulphuric acid which remained), and then all but a 5-cm. thread was rejected. By substituting the beaker of mercury for the beaker of potash, a new mercury seal was introduced outside the thread of potash, and absorption of carbon dioxide was allowed to take place whilst the bubble was oscillated at least ten times from one end of the tube to the other. At the end of this period careful sweeping by manipulation of the mercury thread transferred the bulk of the potash to the left-hand side of the tube (i.e. nearest to the pump), leaving a clean mercury meniscus at the right-hand side. Measurement was then made of the minimal length of the bubble.

The process was repeated, and a 10-cm. thread of alkaline triacetylpyrogallol was added to the potash present. Absorption of oxygen was allowed to take place, again whilst the bubble was rapidly oscillated up and down the length of the tube. Once again the tube was swept clean and all absorbent transferred to the broadened end of the capillary tube, leaving a clean meniscus on the right-hand side. After measurement of the bubble, the whole apparatus was flushed through twice with dilute sulphuric acid, followed by successive rinses with water, and a final rinse which left the walls moistened with 5 per cent. sulphuric acid.

The following comments on the method are made in the light of previous work in which other modifications of the Bonnier and Mangin technique have been used. The use of pyrogallol was abandoned in favour of its triacetyl derivative;[1] the production of carbon monoxide during oxygen absorption made the results obtainable with pyrogallol itself inconsistently variable, and hence for the most part unacceptable. In only one stock solution of pyrogallol that was prepared was there freedom from this source of error. No such error was detected at any time with triacetylpyrogallol.

The Fieser (1924) technique of using sodium hydrosulphite (in conjunction with sodium anthraquinone-sulphonate as a catalyst) was also tried and abandoned. It was adequate for the absorption of oxygen but invariably contaminated the mercury. In an apparatus of this kind it is essential to use reagents which will not interfere with the free running of the mercury thread.

Stress must be laid on the need for adequate sweeping of fluid from the walls of the capillary prior to measuring the bubble. The more efficient the sweeping, the shorter will be the bubble; so that, for instance, a high initial reading of the bubble prior to absorption will most certainly affect low values of carbon dioxide, and, to a lesser extent, low values of oxygen if they occur. Adequate time must be allowed for absorption, and it is of some importance that the potash should be made up in well-aerated water, otherwise low oxygen figures may occur, through lack of equilibrium between the oxygen dissolved in the potash and that in the atmosphere undergoing analysis (see Scholander, 1942). The introduction of error from this source is also reduced by keeping the thread of potash short (5 cm.) by comparison with the total length of bubble (about 20 cm.).

In the early stages of the work a complete rinse through was carried out after each use of triacetylpyrogallol, for the presence of even small traces of this reagent gave an enhanced carbon dioxide value and a diminished oxygen value. However, throughout all the experimental work represented in Fig. 2, two gas-analysers were used side by side, and determinations were made separately upon portions of each sample extracted. One determination was made for carbon dioxide alone (using 10 per cent. potash), and another was made for carbon dioxide plus oxygen (using alkaline tri-acetyl pyrogallol). In consequence, it was only necessary to clean the apparatus throughout once daily. In practice, for purposes of washing the apparatus between successive readings, 5 per cent. sulphuric acid was drawn in as far as the bottom of the wide tube (B) and then expelled. This washing was then followed by two or

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[1] The authors are grateful to Prof. T. A. Bennet-Clark for the suggestion that led to the use of this reagent. Both he and Burton and Spragg (1948) have used 1:2:4—triacetoxybenzene; but some 1:2:3—triacetoxybenzene (triacetylpyrogallol) became available to the authors and proved satisfactory and reliable in use.

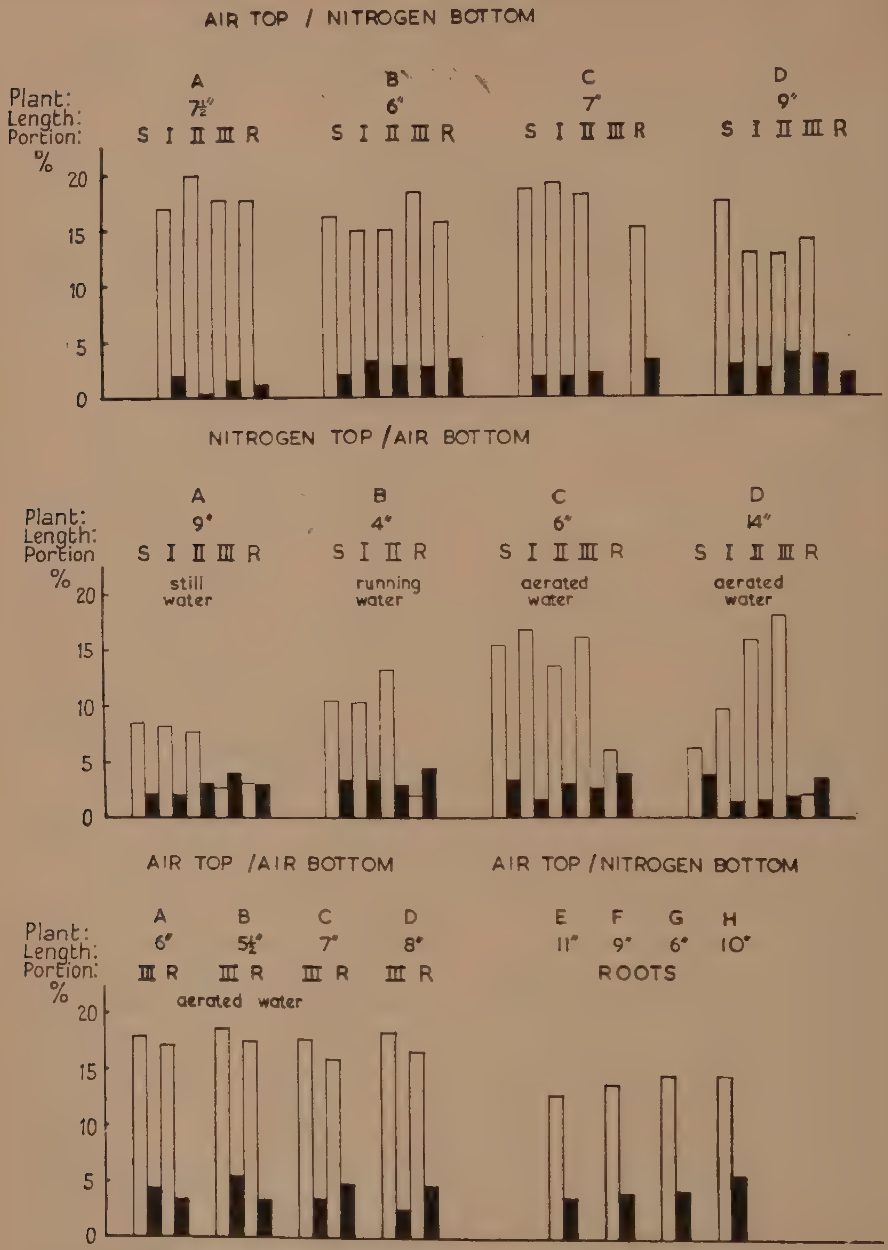

Fig. 2. Composition of internal atmospheres at five different levels (see text) in plants of bogbean, grown under three sets of conditions. Oxygen, white; carbon dioxide, black.

three successive rinses back to the same point with acid, after which the apparatus was ready for immediate use. In this way, not only was there a saving of time, but there was no chance of error on the carbon dioxide determinations, due to residual traces of oxygen absorbent, a feature which had previously been found troublesome.

All analyses were completed within 12 hours of sampling, for it was found that the oxygen content of samples of gas stored overnight over mercury tended to diminish, often quite appreciably. This source of error is hinted at by Conway (1937, p. 86) and is almost certainly due to the presence of oxidizable impurities in the mercury, for which periodic thorough cleansing is an essential.

With regard to the sensitivity of the apparatus, it was found that, in determinations of the composition of laboratory air, the concentration of carbon dioxide, as determined by the gas analyser upon a 100-μl. sample, exceeded that determined by the Haldane apparatus by not more than 0·5 per cent. Allowing for this error in carbon-dioxide determinations, it was not possible to detect any significant difference between the values for oxygen concentration as determined by both methods.

EXPERIMENTAL RESULTS AND DISCUSSION

Fig. 2 shows the composition of the internal atmospheres of the five chosen regions of the plants of bogbean. In the *Air Top/Nitrogen Bottom* series recorded in the histogram it will be seen that, in spite of the variation as between individual plants, oxygen concentration is fairly high throughout all the tissues and does not fall below 12·5 per cent. even in the root system. There is no good evidence of a straightforward diffusion gradient from shoot to root in these plants; and since the differences observed (see, for example, plant *B* of this series) fall well outside the range of experimental error of the determinations, they must be taken to indicate either that a simple gaseous diffusion is not the only mechanism involved in oxygen transfer within the stem, or else that, in spite of all the precautions observed, the later samples in any one 30-minute extraction period may have become modified by atmospheric contamination or by respiratory oxygen loss. The sub-atmospheric concentrations recorded lead one to suppose that atmospheric contamination is the least likely cause of variation.

In plants *A* and *C* mischance vitiated the results for shoot and portion III respectively, whilst the result for roots of *D* was rejected because the air sample had to be stored overnight and gave a low reading in oxygen. Nevertheless, the trend of events is well borne out by such results as were available from earlier determinations. For example, shoot-air analyses gave figures of 18·0 per cent. oxygen to 1·4 per cent. carbon dioxide; and again, 17·5 per cent. oxygen to 2·6 per cent. carbon dioxide. The atmosphere from portion III on two earlier occasions showed 16·5 per cent. oxygen to 1·4 per cent. carbon dioxide, and 16·5 per cent. oxygen to 2·8 per cent. carbon dioxide, respectively. Replications on root atmospheres are to be seen in the lower half of Fig. 2; it is plain that root oxygen varied between 12·5 per cent. and 17·5 per cent. and was thus never likely to be limiting.

In considering the reversal series *Nitrogen Top/Air Bottom*, a clear demonstration is given of the effect of the availability of external oxygen upon its concentration inside the plant. In the case of plant *A* of this series, the lower part of the plant was immersed in still water; in the second plant *B* it was immersed in running water; in plants *C* and *D* of the series the water was well aerated by a bubbler, which operated throughout the whole of the experiment. The effect upon the oxygen concentration of leafy shoot and stem is striking, though more so in the 14-in. plant (*D*), for which a clear diffusion gradient is shown from section III upwards to the shoot. Most striking of all in this series, however, is the fact that the concentration of oxygen in the roots at no time exceeded 6 per cent., whilst in three cases out of four the concentration was at or below 3·1 per cent. This suggests, firstly, that the roots themselves take up only a very limited amount of oxygen (or none at all), and, secondly, that the oxygen which does reach the roots does so by way of the shoot. In other words, under these conditions oxygen gains access to the cortex, builds up a diffusion gradient to the shoot, and is re-exported from there, presumably down the stele, to the root.

In the *Air Top/Air Bottom* series only portion III and roots were considered, and it was obvious that, under these circumstances, there was little barrier to diffusion of oxygen within the plant. In both root and portion III the presence of oxygen at or about the 17·5 per cent. level of concentration suggested that the only serious barrier to diffusion occurred at the surface of the plant.

It would appear from the first two series that whilst roots of plants under normal conditions and in darkness show an oxygen content in their internal air space system which rarely falls below 12·5 per cent., reversal of these conditions lowers root oxygen, even under optimal conditions of bottom aeration, to a maximum concentration of 6·0 per cent. (and usually much less than this figure). This can best be explained by supposing that gas movement takes place independently in stele and cortex. Anatomical evidence confirms the absence of continuity of air space between the tissues of emergent roots and the tissues of the cortex through which they pass; and from this point of view it would be reasonable to assume that the roots derive the bulk of their oxygen from the shoot via the stele. So that, in this series, with nitrogen around the leafy shoot, and air or aerated water around the stem, oxygen can pass into the cortex, but before reaching the roots it must pass upwards to the shoot and be re-exported down the stele. For this view to hold, there must be some effective barrier to gaseous diffusion between cortex and stele; in point of fact an endodermis can be detected at a distance of little more than 5 mm. below the growing-point and downwards into the stem. There is therefore some justification on anatomical grounds for assuming the existence of such a barrier.

With *Air Top/Nitrogen Bottom* conditions, it seems that gas movement can take place downwards through the air spaces of both cortex and stele, although it has already been stressed that so far there has been little evidence found of a straightforward diffusion of oxygen. The possibility must be borne in mind that a non-diffusional mechanism, of the type described by Brown (1948) for *Cucurbita pepo*, may account in part for the discrepancies of Fig. 2, and it may even be that cortical and stelar gas movements are completely independent, and are not necessarily motivated in the same way.

One further experiment may be mentioned here, in which a plant was left in the greenhouse with its shoot exposed to the external atmosphere, but with its lower stem and roots sealed into an 800-ml. flask containing about 250 ml. of culture solution with an atmosphere of nitrogen above. The plant was first thoroughly washed before it was sealed into the chamber. For comparison, a similar experiment was set up, using a plant in which the mud still adhered to the roots in considerable quantity. At daily intervals 10-ml. samples were withdrawn from each chamber and were analysed in the Haldane apparatus. Any nitrogen remaining from the determination was replaced in the chamber. Over a period of 14 days the oxygen content of the first chamber rose from 0·04 per cent. (the value for the cylinder nitrogen) to 1·3–1·5 per cent., and never at any time during this period did it vary from these limits. Had bacterial action been effective during this fortnight, one would have expected the concentration to diminish appreciably from this figure; but it did not do so. In the second chamber, with a quantity of mud present, the oxygen rose to a maximum of 0·2 per cent. in 2 days, and then diminished fairly rapidly to zero as bacterial activity became prominent. Thus, where bacterial activity was not apparent there was a positive movement of oxygen into the nitrogen-filled chamber. It is worth noting here that whilst the concentration of oxygen in roots under *Air Top/Nitrogen Bottom* conditions has never been observed to fall below 12·5 per cent., the oxygen concentration in the atmosphere above the fluid surrounding them remained fairly constant at this considerably lower level of 1·3 to 1·5 per cent.

It may be recalled that van Raalte (1943) has drawn attention to the fact that around the roots of the salt marsh plant *Salicornia herbacea* L. there is frequently to be seen evidence of oxidative processes at work. It is by no means certain from our present experiments that the movement of oxygen into the gas space of the lower chamber is accomplished by the roots alone. There may be with the present arrangement a casual leakage across a rupture in the epidermis for example. Nevertheless, the establishment and maintenance of a steady but low concentration in the nitrogen atmosphere of the chamber over a period of 14 days would seem to argue in favour of a controlled secretion rather than of an uncontrolled leakage. Meanwhile a fuller investigation is in progress.

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Factors controlling Flowering in the Chrysanthemum

II. DAY-LENGTH EFFECTS ON THE FURTHER DEVELOPMENT OF INFLORESCENCE BUDS AND THEIR EXPERIMENTAL REVERSAL AND MODIFICATION

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WITH THREE PLATES AND ONE FIGURE IN THE TEXT

SUMMARY

1. Inflorescence buds, produced by vernalized Chrysanthemum plants in long day, cease to grow, and die at an early stage if maintained in long day, but will produce open flowers if transferred to short day. Dissection of such buds reveals that development does not proceed beyond the formation of the bare receptacle and no florets are initiated, while inflorescence buds produced in short day have almost completed floret initiation when they become macroscopically visible.

2. Inflorescence buds produced in long day can be induced to complete their development in long day by:

(*a*) removal of all lateral shoots, and

(*b*) by re-rooting the inflorescence itself, leaving only a number of bracts on its axis.

3. Inflorescence buds produced in short day can be inhibited from developing by

(*a*) transfer to long day,

(*b*) transfer to low light intensity in short day, and

(*c*) application of auxin paste.

All three methods of inhibition become progressively less effective with the advancing development of the bud.

4. The latest stage at which development was found to have been arrested was that of ovule formation.

5. Heights of plants were determined at budding and when the flowers had started to open; marked differences due to length of day were found.

6. Teratological effects noted in buds, exposed for extended periods to long day, included formation of bracts on the receptacle (the absence of which distinguishes the subtribe *Chrysantheminae* of the Compositae to which the Chrysanthemum belongs) as well as secondary inflorescences, petaloid stamens, &c.

7. The results are discussed in relation to known effects of auxin on vegetative growth and reproduction.

INTRODUCTION

In the previous paper of this series (Schwabe, 1950) the important role of temperature on the time of inflorescence bud initiation in the Chrysanthemum has been discussed.[1] The further development and opening of the inflorescence buds after initiation, however, appeared to depend largely on the length of day given. Under natural conditions, i.e. without manipulation of day-length, and depending upon the lateness of the Chrysanthemum variety used, such potential inflorescence buds are produced normally throughout the summer months from June to September. In the then prevailing long days these buds fail to develop and usually abort after some time. If such plants are transferred to short day, however, open flowers are formed from the potential inflorescence buds (cf. Fig. 1, loc. cit.).

Previous work on the further development of flower or inflorescence primordia formed after photoperiodic induction yielded results depending on the species used. Borthwick and Parker (1938) showed that in the soybean a further period of short day is required after initiation to ensure normal flower development. In *Xanthium*, a single short-day cycle will lead to the production of an abnormally high proportion of male inflorescences while four cycles are almost sufficient for normal flowering, but the rate of floral development depends even then on the number of cycles given (Naylor, 1941; Mann, 1940). Murneek (1940), working with the long-day plant *Rudbeckia bicolor*, showed that transfer to the unfavourable day-length of plants given a period of long-day induction may also check or delay their further development and abnormal flowers may be produced. Eguchi (1937) drew attention to the fact that in a number of species the optimum day-length may change with the stage of development of the reproductive organs, and he has designated plants as SS, LL, SL, or LS, according to the sequence of their optimum day-lengths for flowering, fruiting, &c., but the adequacy of such a scheme requires confirmation.

Bormann (1939), working with a large number of different plants, investigated the environmental conditions necessary to cause reversion of inflorescences to the vegetative condition and their rooting responses. As long ago as 1939 Cholodny had suggested that there might be an interrelation between flowering and growth hormones. While the work to be reported in this paper was in progress, Green and Fuller (1948) published the results of some experiments on the effect of growth hormones on cut inflorescences. They found delaying and inhibitory effects similar to those which will be described below. Also, Bonner (1949) and Bonner and Thurlow (1949) and Čajlachjan (1948), as well as Harder and Van Senden (1949), have reported inhibitory effects of 'auxins' on the actual photoperiodic induction, but usually only when the plants were kept near their critical day-lengths. They also successfully employed 'auxin antagonists' to counteract the inhibitory effects. Galston (1947) used triiodobenzoic acid and interpreted its effects as due to auxin inactivation. Wareing (1949) suggested that day-length effects on the vegetative development of *Pinus* and other woody plants were due to auxin differences. Leopold and Thimann (1949) believe that there may be an optimum auxin level for flower initiation, at least in long-day plants. Resende (1949) postulated a balance between auxin and anti-auxin.

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[1] Since that paper was published the author's attention has been drawn to a paper by Drs. J. T. Martin and F. Tattersfield (*Ann. Appl. Biol.* **21**, 682–90, 1934) in which a similar cold requirement for flowering has been described for a different species of Chrysanthemum, *C. cinerariaefolium*.

THE DEVELOPMENT OF POTENTIAL INFLORESCENCE BUDS IN CHRYSANTHEMUM MAINTAINED IN LONG DAY

Before discussing the experiments designed to influence the behaviour of potential inflorescence buds, a description of the normal course of their development in long day will be presented. Such buds become macroscopically distinguishable when rather smaller in size than equally distinguishable inflorescence buds produced in short day. Almost immediately after the bud becomes visible, lateral buds in the axils of the youngest uppermost leaves usually 3–4) begin to grow out into vegetative shoots, while the inflorescence ud itself ceases to enlarge further. In short day the corresponding lateral uds also grow out, but develop directly into separate inflorescences. In continued long day the terminal apices of the laterals invariably form potential nflorescence buds in turn after some time. This course of development may e repeated time after time—one plant kept in long day for almost 2 years ran hrough six such cycles.

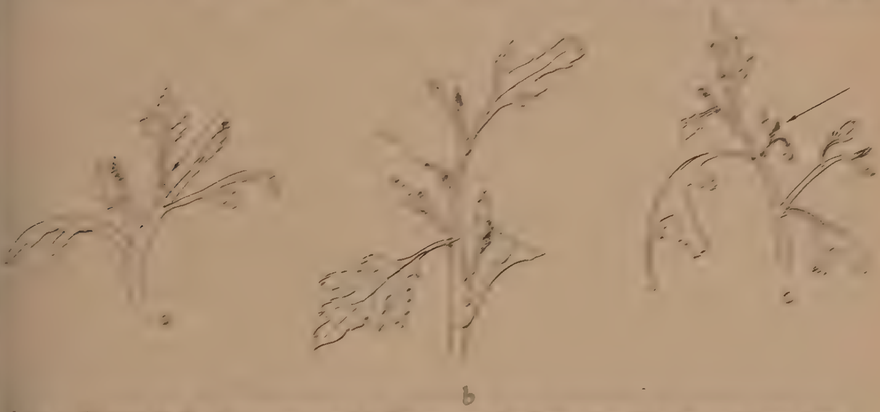

FIG. 1. Stages in the suppression of inflorescence buds produced in natural long day.

The degree of suppression of these inflorescence buds varies between different varieties and also within a single variety. Within the variety 'Indian ummer', used in most of the work reported here, variability in this respect as very small. In other varieties a graded series in the average degree of uppression may be found ranging from the complete absence of any trace of he terminal apex to a definite inflorescence bud surrounded by numerous racts. In Fig. 1 some of these stages are shown diagrammatically. In the periments described below the stage reached by the bud has always been hat shown in Fig. 1, *c*. Dissection of nearly two dozen such buds at various mes revealed that development always ceased when the receptacle had been fully formed, but before any florets had been initiated. In Fig. 2 the bare receptacle dissected from a vernalized plant budded in long day is shown; Fig. 3 represents an apex from a plant budded in S.D. at the time it became macroscopically visible. Here floret initiation has progressed and is nearing completion.

For a fuller understanding of the conditions determining the development of these buds (whether produced in L.D. or S.D.) attempts have been made—(*a*) to induce buds to complete their development in long day, and (*b*) to inhibit their development in short day.

Production of open flowers in long day

The inhibition of the terminal bud produced in long day concomitantly with the development of vegetative lateral shoots bears a striking resemblance to phenomena described as 'apical dominance'. Chronologically it seems that first the terminal growing-point loses its apical dominance completely as soon as it becomes irreversibly reproductive. The 3–4 laterals growing out then appear to assume 'dominance' over the original main apex. Thus it seemed that the actual suppression of the terminal potential inflorescence bud might be due to their influence. Consequently it was thought possible that this bud might be induced to continue its development if it could be isolated from the influence of these vegetative lateral shoots.

Two methods of isolation were attempted: (1) continuous removal of all lateral or secondary shoots on the whole plant while maintaining it in long day, and (2) removal of the inflorescence bud itself by severing it above the last true leaves, but leaving on as many bracts as possible to enable photosynthesis to proceed. These bracts bear no buds in their axils and no adventitious shoots are produced. Such inflorescence cuttings were then rooted and grown on in long day.

Both methods proved successful and open flowers were produced in long natural day extended to more than 16½ hours by additional illumination with incandescent light (Figs. 4 and 5). Three varieties were tested by the first method: 'Indian Summer', 'Cossack', and 'Godfrey's Gem'; in the second 'Cossack' was replaced by 'Poitevine'. However, the development of these buds was considerably slower than it was in control plants transferred to short day. Some took several months to open under these conditions. Hence a residual inhibitory effect must be ascribed to the effect of long day perceived by the leaves or bracts left on the plant or cutting. In both methods a number of inflorescence buds was usually lost owing to attack by parasitic fungi during the prolonged period of development.

Inhibition of development of inflorescence buds produced in short day

1. *Transfer to long day*. As was mentioned before, the young inflorescence bud produced in short day has usually entered or completed the stage of floret initiation by the time it becomes visible macroscopically. It appeared of interest, therefore, to discover whether it would be possible to arrest the

development of such buds; i.e. at a later stage than that at which untreated long day buds cease further development. An attempt was also made to determine the stage at which such arrest would no longer be possible. The first procedure designed to arrest these inflorescences was to transfer plants budded in 8-hour days to long day or continuous light either immediately or at some time after the macroscopic appearance of the buds. The long day consisted of 8 hours' full daylight followed by 8½ hours' low-intensity artificial light from incandescent lamps (approximately 25 ft. c.) and 7½ hours' darkness; when continuous light was given 8 hours' daylight were followed by 16 hours' low intensity artificial illumination. The results of a preliminary experiment are shown in Table I. Some representative plants are shown in Fig. 6.

TABLE I

Preliminary experiment on inflorescence bud inhibition by long day. Variety 'Indian Summer'

Length of day	Time of transfer	Number of replicate plants	Number of buds suppressed Main bud	Lateral 1	2	3
24 hours	Immediately after budding	7	6	1	1	1
24 hours	Up to 7 days after budding	2	0	0	0	0
16½ hours	Immediately after budding	3	3	3	3	3
16½ hours	Up to 14 days after budding	3	1	1	1	1

Although only a few plants were used in this preliminary experiment, it clearly demonstrates the inhibitory effect of long day. This effect has been described previously by a number of authors (Hamner, 1938; Post, 1932; Behrens, 1949), but usually the inhibition has been regarded as due to sub-optimal induction. This is not likely to apply in the present case as the plants used were derived from short-day parents, vernalized in short day, and grown in short day continuously until the final transfer (i.e. usually they received 2 months or more of short day). Delayed transfer, i.e. of buds allowed to develop for some days in short day after their macroscopic appearance, is much less effective. There is also a large difference between the main terminal bud and the laterals, the main bud being much more readily inhibited than the first 1–3 laterals. There also appears to be a difference between two long-day treatments, 24 hours and 16½ hours, the latter being somewhat more effective in arresting inflorescence bud development. These differences are seen more markedly in a second experiment. The plants used were again derived from vernalized short-day cuttings of the variety 'Indian Summer' and again transferred to long day at a comparatively late stage in their development, i.e. after the macroscopic appearance of the inflorescence bud. Two sets of controls were grown; one was maintained in short day throughout, the other transferred to long day but subsequently returned to short day at different times, the average time spent in long day being 1 month. The object of this control was to discover whether, after a prolonged period of long day, re-transfer to short day would still allow all buds to complete their development. The delay in opening serves to measure the degree of inhibition where this is not complete;

the first appearance of the petals below the scarious involucral bracts was noted as a convenient measure of the time of opening of the 'flowers'. The results are shown in Table II. Again the delaying effect of long day on flower opening is clear. The fact that 8 out of 11 main buds in 16½-hour day were totally stopped and none in 24-hour day is rather surprising and not easily explained, though a similar effect was noted in the preliminary experiment.

TABLE II

Effect of transfer to long day of plants budded in short day.
Variety 'Indian Summer'

Treatment	Number of replicates	Number of buds suppressed	Days to appearance of petals after budding (suppressed buds omitted)	Mean Height when budded (in cm.)	Mean Height increase after budding
S.D. controls (not transferred)	13	0	33	26·2	27·6
Transferred to 24-hour days					
Main bud	10	0	60	28·0	58·5
First lateral bud	10	0	58	—	64·5
Second lateral bud	9	0	59	—	64·5
Transferred to 16½-hour day					
Main bud	11	8	45	27·2	38·0*
First lateral bud	10	0	63	—	50·8
Second lateral bud	8	0	68	—	52·7
Transferred to 16½-hour day after budding then returned to 8-hour day after an average of 30 long days†	12	1	51	30·5	48·3
Standard deviation (per single plant)					
Short-day controls	—	—	± 2·02	± 4·12	± 3·64
Transferred plants main bud	—	—	±15·7		±10·3
Transferred first lateral bud	—	—	±10·6		± 7·27
Transferred second lateral bud	—	—	±12·9		± 8·85

* This includes eight suppressed buds.

† These plants were treated under similar conditions but at a different time from the other treatments.

In addition to these data the heights of the plants were measured from soil level to the top of the bud. The measurements were made at the time of budding and again when the petals showed—in the suppressed buds, when growth had ceased. They are presented in the same table. There are large differences in height increase (i.e. elongation of existing internodes, since no new nodes can be formed after budding). Here it would appear that continuous light leads to an even greater degree of elongation than 16½-hour day.

Finally, those buds which failed to open were sectioned and the stage determined at which development was brought to a standstill. Five easily recognized stages in the development of the floret have been used to classify the buds dissected:

(1) the 'early cup stage': beginning from the time when the floret primordium is formed as protuberance on the receptacle;

(2) 'late cup stage': beginning from corolla tube initiation;

(3) 'anther stage': beginning from early anther formation;

(4) 'gynoecium stage': beginning from early gynoecium formation;
(5) 'ovule' formed in ovary.

Of the buds which failed to open three were arrested in stage 2, two in stage 3, one each in stages 4 and 5, while one was lost in dissection. Again the last stage at which this arrest took place was the formation of the ovule in the gynoecium, though most were stopped before reaching this stage.

The similarity of the long-day and apical dominance effects noted above as well as the marked differences in internode elongation suggested strongly that auxin effects were involved in this inhibition of inflorescence development in long day. In order to gain further evidence on the point two more experiments were set up:

(*a*) Placing plants into environmental conditions which among other effects are known either to increase the natural auxin content above the normal level, or the sensitivity of the plant to its action, and
(*b*) the direct application of β-indolyl-acetic acid paste.

2. *Shading in short day.* The first method used to arrest the development of inflorescence buds in short day was a drastic reduction in light intensity in short day. This it was hoped would result in an increased internal auxin concentration. Plants which had almost completed the first stage of bud development, but a day or two before the buds were actually visible macroscopically, were transferred to low light. This was achieved by surrounding the containers in which they were grown by cylindrical shades of black material, the open tops of the cylinders being covered with a light muslin cloth. Photoelectric measurements indicated a reduction in light intensity to about 10–15 per cent. of full daylight during their 8-hour period of illumination. A second group of plants was allowed to remain in full light until their buds had become fully visible (about 2–3 mm. in diameter); they were then transferred into the shade.

All eleven replicates of the set shaded early showed complete arrest of the main bud immediately after its macroscopic appearance. The peduncles of the inflorescences bore large bracts and the uppermost axillary branches grew out in the same manner as in plants transferred to long day (Fig. 7). The main buds of those plants transferred to shade at a later stage were not arrested (Fig. 8). The effect of shading can also be seen from the state of the lateral branches at the end of the experiment. Numbering these downwards from the main bud the numbers which were found to have become vegetative are shown in Table III.

TABLE III

Number of vegetative laterals produced on budded S.D. plants

Treatment: Light conditions in short days	Number of replicates	Number of plants with vegetative laterals				
		1st lat.	2nd lat.	3rd lat.	4th lat.	5th lat.
Early shade . .	11	1	1	6	11	11
Late shade . .	6	0	0	0	1	1
Full light . . .	9	0	0	0	0	0

Hence early shading under short-day conditions is seen to have the same effect as transfer to long day.

3. *Application of β-indolyl-acetic acid paste in short day.* Preliminary experiments with β-indolyl-acetic acid paste had shown that the effectiveness of auxin application depended on the stage of development of the bud. Where the bud had reached a somewhat more advanced state than that at which it first becomes macroscopically distinguishable β-indolyl-acetic acid application failed to have any effect.

In one experiment 20 older buds were treated with auxin paste. In half of these the paste was applied some distance below the flower-head on the inflorescence stalk; the remainder were ringed with paste just beneath the involucral bracts. The diameter of the buds ranged from 5 to 10 mm. All the buds treated low on the flower-stalk opened normally, the time required depending on their original size. In the second set the four smallest buds treated were arrested. As had been observed in some plants arrested by transfer to long day, the young florets were the first members to show signs of injury and death, beginning with the ovules which had just been formed. The larger buds showed no signs of injury and opened normally. Also, as was to be expected, the effect of applied β-indolyl-acetic acid paste was localized to the treated bud. The method of application by ringing the peduncle close to the involucral bracts is the same by which Dettweiler (1942) treated *Tagetes patula* inflorescences. However, if the peduncle were allowed to elongate under short-day conditions until it had attained a sufficient length to be treated, floret initiation in the bud would have reached too advanced a state for the auxin treatment to have any effect. Hence it was decided to transfer the experimental plants to long day as soon as their inflorescence buds became evident. This treatment ensured maximum rate of peduncle elongation while at the same time arresting the morphological differentiation of the florets themselves to a large extent. As soon as the peduncles were long enough auxin paste—and pure lanolin in the case of the controls—was applied to the uppermost inflorescence buds. Two days after application of the paste the plants were returned to short day. A high concentration of auxin (500 p.p.m.) was used in order to maintain a continuous supply for some time. A similar set of plants was treated and maintained in long day. In this treatment, however, all lateral buds were removed, so as to exclude the 'dominance' effect exerted by these (cf. above); the auxin paste was applied to the cut stumps of the 3–4 uppermost laterals. In addition a ring of paste was again applied below the terminal bud. Lanolin controls were used again as in the short-day set. The pastes were renewed from time to time since continued elongation of the peduncles, &c., dispersed the ring of lanolin or auxin paste. At the end of the experiment all buds which had been arrested were sectioned by hand and the state of development of the florets determined. In Fig. 9 a terminal bud arrested by β-indolyl-acetic acid paste is shown.

The results shown in Table IV clearly demonstrate the inhibitory effect of auxin. As a further control treatment about a dozen vegetative apices

TABLE IV

Effect of ringing peduncles of inflorescence buds with β-indolyl-acetic acid paste (500 p.p.m.) in short day and long day. Variety 'Indian Summer'

Treatment and final day-length	No. of replicates	Mean No. of long days pre-treatment	No. of buds suppressed	Days to appearance of petals after budding (omitting suppressed buds)	Average Height when budded (cm.)	Height increase during long-day pre-treatment	Height increase after treatment (cm.)
Short-day lanolin controls	58*	16	(1)†	37	27·0	25·7	12·7
Short-day auxin:							
Main shoot	13	19	10	39	26·7	29·9	10·1
First lateral	13	—	10	—	—	—	—
Second lateral	12	—	10	—	—	—	—
Third lateral	13	—	10	—	—	—	—
Fourth lateral	11	—	9	—	—	—	—
Long-day lanolin controls	10	15	0	43	27·5	26·4	17·4
Long-day auxin:							
Main shoot only	11	15	10	34 (1 plant)	27·1	27·2	15·0
Standard deviation (per single observation)	—	—	—	±10·2	±5·70	±8·62	±4·09

* Sum of all main and lateral buds treated on 11 plants.
† Third lateral.

TABLE V

Stage at which buds were finally arrested, determined by hand sectioning. Numbers of buds falling into each of the five classes are given

Treatment	No. of replicate plants	No. of buds treated	Stage at which bud development was arrested					Lost
			Early cup stage	Late cup stage	Anther stage	Gynoecium stage	Ovule stage	
Short-day lanolin controls	11	58	0	0	0	0	0	1
Short-day auxin	13	64	5	9	5	18	11	1
Long-day lanolin controls	10	10	0	0	0	0	0	0
Long-day auxin	11	11	1	0	2	3	1	3

(vernalized and unvernalized plants in short day, and vernalized plants in long day) were also ringed with auxin paste, after removal of some young leaves, to determine whether the rather high concentration of β-indolyl-acetic acid used would also arrest the growth of vegetative apices. However, none of these apices was arrested; all resumed normal growth though they were delayed somewhat.

Again plant height measurements were taken and are shown in Table IV, but here the differences are quite small. In Table V the stage of development reached by the arrested buds has been recorded. Again the most advanced stage of development reached by any of these buds was that of ovule formation; and it appears that this is the last stage at which development can be arrested by the methods used here.

TERATOLOGICAL PHENOMENA

In these experiments a number of teratological modifications of the inflorescences, &c., occurred with such regularity that it appears worth while to give a brief description of them.

1. *Floral bracts*

Among the Compositae, the absence of bracts on the receptacle (other than the outer involucral bracts) is regarded as one of the distinguishing characters of the subtribe Antheminae—Chrysantheminae (Engler and Prantl) or Chrysantheminae (Rendle) including the genus *Chrysanthemum*. However, in all open inflorescences exposed to long day, whether subsequently returned to short day or not, large numbers of bracts have been found, confirming earlier unpublished observations by M. Holdsworth. These bracts resemble the innermost papery involucral bracts normally surrounding the inflorescence, and appear to subtend florets in their axils. This subtribe character then can be easily modified by manipulation of the environment. The phenomenon itself seems to be related to the 'phyllody' of *Kalanchoe blossfeldiana* demonstrated by Harder and co-workers (Harder, 1948).

2. *Secondary inflorescences*

In all plants which had budded in long day and were forced to produce open 'flowers' in this day-length, subsidiary inflorescences were produced inside the ring of involucral bracts surrounding the original inflorescence rather like a 'hen and chicken' daisy. In Fig. 10 one of these multiple inflorescences is shown. For the sake of clarity the primary florets have been removed, leaving only the secondary inflorescences. Each of these secondary inflorescences originated from the growing point of a single floret which had formed a short pedicel inside the normal corolla tube. Tertiary inflorescences were never found. Such secondary inflorescences are also found occasionally under natural conditions in other families such as the Dipsacaceae (e.g. *Cephalaria tartarica*).

When inflorescence buds were rooted after being cut off from the 'parent' plant, the same phenomenon was observed, and here a number of instances occurred where even a leafy shoot grew out of the corolla tube, subsequently forming one or more inflorescence buds. One of these is shown in Fig. 11.

Finally a few other abnormalities were observed in plants flowering in long day, such as petaloid stamens, enations on petals and petaloid stamens, and intermediate stages between corolla tubes and floral bracts, &c.

Konovalov (1949) described a number of such abnormal inflorescences on long-day plants found in nature. He ascribes the effect to the autumnal short day on late flowering specimens.

DISCUSSION

The results presented can be summarized very briefly: Chrysanthemum inflorescence buds, produced in long day, normally cease to develop and die if maintained in this day-length. They can be made to complete their development:

(1) by transfer to short day;
(2) by removal of all lateral shoots in long day; and
(3) by re-rooting the isolated inflorescences in long day.

Inflorescence buds produced in short day can be inhibited from completing their development:

(1) by transfer to long day;
(2) by shading in short day; and
(3) by application of auxin paste.

All three inhibitory treatments are effective only in early stages of bud development. The latest stage at which buds have been found to be arrested is that of ovule formation.

All these observations are compatible with the suggestion that excess auxin may have an inhibitory effect on inflorescence development. Evidence for higher auxin content in plants exposed to long day is rather scanty, but Čajlachjan (1937) and later Leopold (1949) have advanced some evidence to this effect. Shading of course exerts at least two effects: it reduces carbohydrate level and also increases either the internal auxin content or the sensitivity of the plant to its action (for a general review see Galston, 1950). The apical dominance effects referred to are also known to be due to auxin (Thimann and Skoog, 1933, 1934).

The effect of bud size (i.e. the progressive ineffectiveness of all three inhibitory treatments, as the stage of development of the bud advances) noted in the present experiments may perhaps also be an indication that inhibition by application of auxin paste does not involve a different mechanism from that of long day, shading, &c. Dettweiler (1942) also noted this size effect and failed to stop the development of rather older buds of *Tagetes patula*.

The observation that inflorescence buds on the uppermost 1–3 lateral shoots of the Chrysanthemum (variety 'Indian Summer') are less easily arrested by transfer from short day to long day than the main bud may perhaps be explained by the absence of secondary laterals on the primary set. Hence these are less subject to suppression than is the main bud itself.

The large differences in internode elongation between short-day controls and plants transferred to long day also supply indirect evidence of differences in auxin content. Even the manner in which long day is given may result in different degrees of elongation. In one experiment Chrysanthemum plants were given two types of long-day treatment: (i) 8-hour daylight and 8-hour low intensity artificial light; (ii) the other group received nearly all 16 hours as natural summer light. The main internode length of set (i) was 1·12 cm.; set (ii), 0·92 cm. The *t*-value for the difference is 4·15 (t_{20} for 0·001 = 3·85).

It would appear, therefore, that the usual method employed in day-length experiments of subjecting all plants to the same period of bright light and supplementing this by weak light in the case of long day, with the aim of equalizing assimilation between plants given short day and long day, may still exert other differential effects (e.g. on the auxin metabolism) which may be of considerable morphogenic importance. Borthwick, Hendricks, and Parker (1948) found that in barley, at least, the phenomenon of internode elongation could not be separated from that of flowering itself. Murneek (1940), on the other hand, succeeded in arresting stem elongation in *Rudbeckia* after the inflorescence had been initiated, but flower-stalk inhibition was not complete. He suggested differential growth-hormone production without accumulation of reserves as possible cause for these day-length effects. In this connexion attention may perhaps be directed to some of the work on the light requirement of short-day plants (Snyder, 1940; Mann, 1940) where light of low intensity was shown to be insufficient to provide the precursor for the train of reactions leading to the production of the flowering hormone. It seems entirely possible that partly at least we may be concerned here with auxin effects more or less indirectly influencing flower induction.

However, all this must remain in the realm of conjecture in the absence of any valid growth-hormone determinations in the plant material. Little need be said about the intrinsic difficulty of obtaining unequivocal values for the effective hormone content in the plant in the presence of inhibitors (Stewart, 1939), inactivators, and inhibitors of the inactivators (Tang and Bonner, 1948). Attempts by the author to determine auxin content of leaves have merely succeeded in demonstrating the presence of inhibitors which completely mask any auxin effect.

If it be assumed that the inhibitory effects seen *are* due to auxin, one may consider the question of its source. Snow (1929, 1937) showed that the young leaf primordia provide the major source of the auxin issuing from the apex. Experiments on leaf abscission indicate the possibility of replacing the effect of the lamina by application of β-indolyl-acetic acid paste (Gawadi and Avery, 1950) and suggest strongly that even adult leaves produce a substance which is related at least to the growth hormones. It seems worth recalling in this connexion that long-day treatment of some deciduous trees delays leaf abscission

(Matzke, 1936). As regards the present experiments the source of the inhibitory auxin in plants budded in long day might then be sought primarily in the young leaves of the vegetative lateral shoots, while smaller amounts would be derived from the older leaves below the bud, the effect moving in a non-polar direction. That older leaves can effect the growth and development of younger members above them has been shown by Ashby (1948), as also by Goodwin (1937).

One other observation requiring explanation is the stem elongation which occurs subsequent to the actual macroscopic appearance of the inflorescence bud. Thus the data presented show that in short day the total height of the plant is doubled after this stage without any increase in internode number. It seems likely that there is a correlation here with the actual state of development of the floret initials and, as has been shown above, the last stage at which it has been possible to arrest the further development of buds is that of ovule formation in the florets. It might be suggested that the auxin required for this considerable elongation of the upper internodes and peduncle is at least partly derived from the florets at this stage. Katunskij (1936) observed similar effects with *Papaver nudicaule* and *P. rhoeas* which he claimed could actually be correlated with definite nuclear divisions in the embryo, &c. Hatcher (1945) made a careful study of the auxin content of anthers and carpels during the development of the rye ear. He pointed out that the maximum stem elongation occurs prior to fertilization. In the Chrysanthemum inflorescences used here fertilization does not take place at all unless the plants are pollinated artificially. However, a more detailed study of the relation between internode elongation and apical differentiation is in progress now. If the suggestion is correct, the loss of sensitivity of the bud to applied auxin, &c., would coincide with the stage when its own production is rising.

In spite of all these suggestive correlations between the probable internal auxin contents, &c., in the author's opinion, it is unlikely that flowering is controlled simply by auxin acting as inhibitor, or even by some optimum level as suggested by Leopold and Thimann. The many successful grafting experiments demonstrate beyond doubt that a positive factor must be involved also, for it would hardly be plausible to regard the 'donor' merely as a 'sink' for the inhibiting substance. Perhaps one might suggest that the positive factor functions indirectly by controlling the auxin level in the plant.

The author is indebted to Professor F. G. Gregory and to Mr. F. J. Richards for stimulating interest and helpful suggestions in the course of this work.

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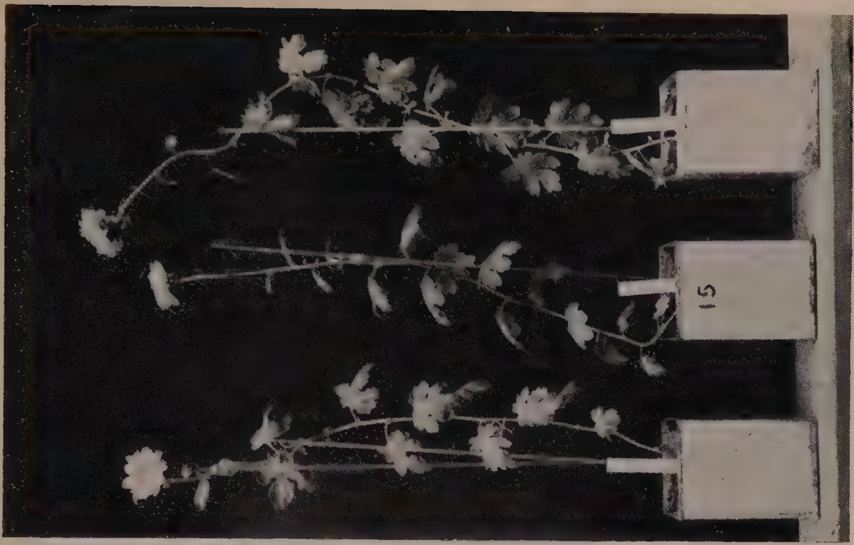

FIG. 4.

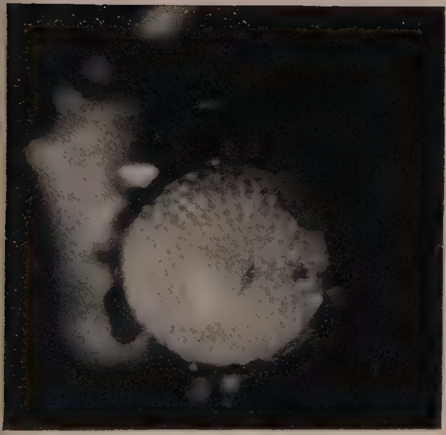

FIG. 3.

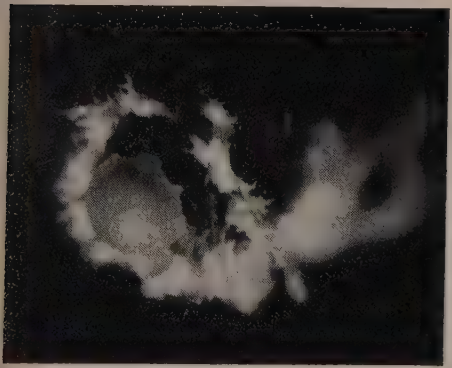

FIG. 2.

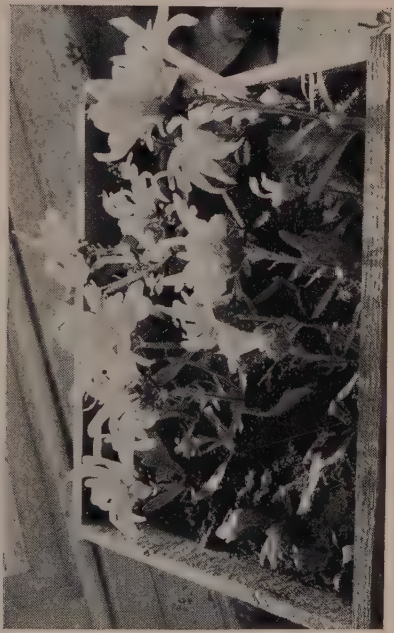

FIG. 5.

W. W. SCHWABE — PLATE I

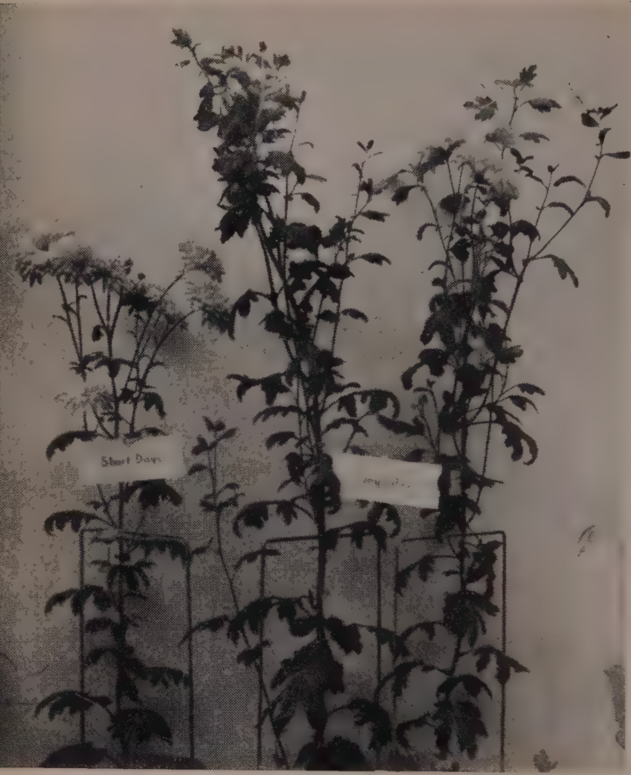

Fig. 6.

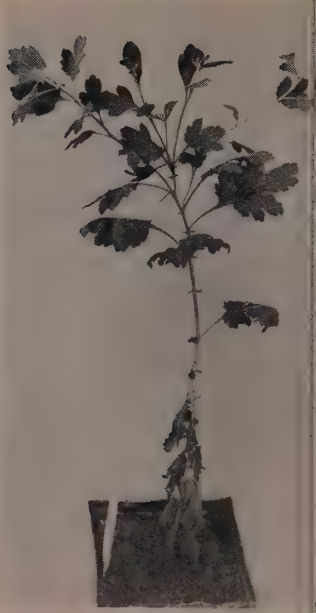

Fig. 7.

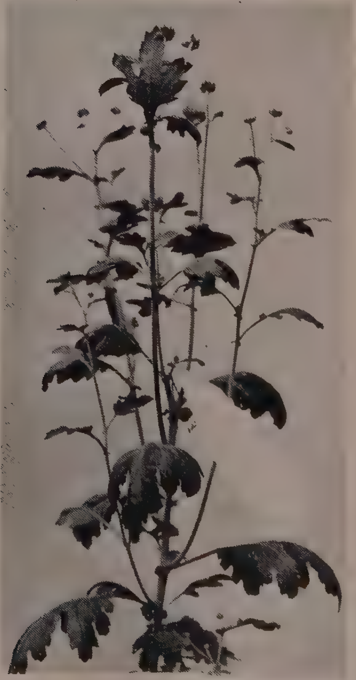

Fig. 8.

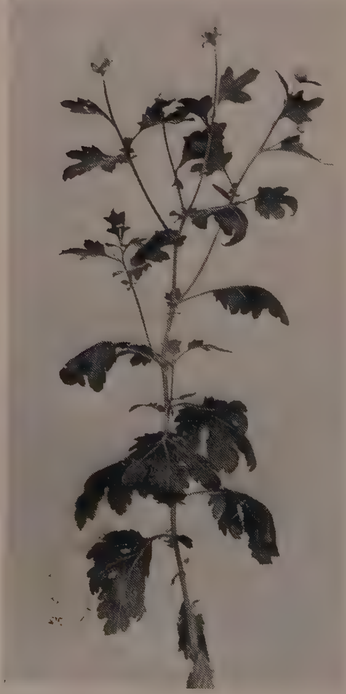

Fig. 9.

W. W. SCHWABE—PLATE

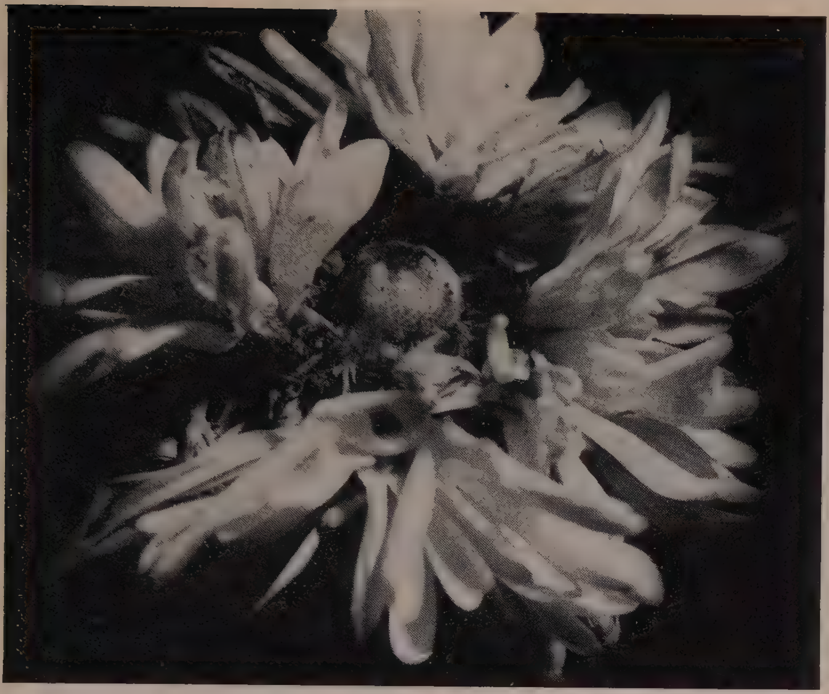

Fig. 10.

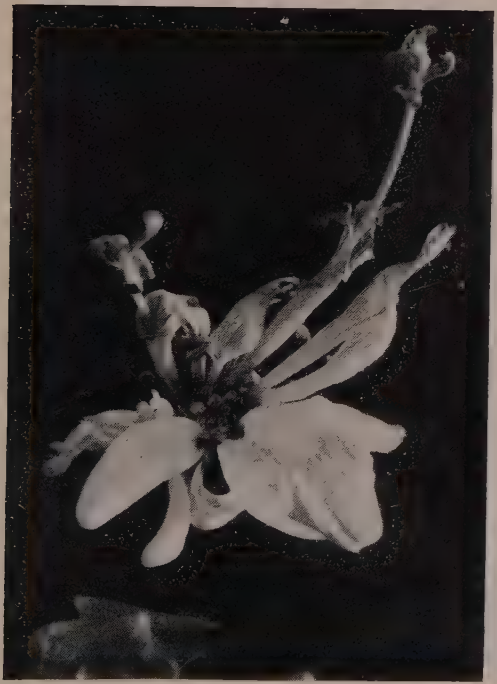

Fig. 11.

W. W. SCHWABE — PLATE III

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DESCRIPTION OF PLATES

Figs. 2 and 3. Inflorescence bud apices dissected at the time when they became macroscopically visible. (2) From vernalized plant in long day showing bare receptacle; (3) from vernalized plant in short day showing floret initiation nearing completion.

Fig. 4. Open inflorescence produced in long day by continuous removal of lateral shoots.

Fig. 5. Open inflorescences produced in long day by re-rooting the severed inflorescences.

Fig. 6. Transfer of budded plants to long day, left to right. Short-day control. Plants showing complete arrest of all inflorescence buds. Plant transferred too late to effect complete arrest.

Figs. 7 and 8. Effect of shading in short day. (7) Early transfer showing complete arrest of inflorescence buds; (8) delayed transfer buds not arrested.

Fig. 9. Terminal bud arrested by treatment with auxin paste.

Fig. 10. Open inflorescence produced in long day showing secondary inflorescences; for the sake of clarity the primary florets have been removed.

Fig. 11. Open inflorescence produced in long day by re-rooting separately, showing shoots growing from corolla tubes.

A Note on the Determination of Alcohol in Potato Tubers

J. BARKER

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SUMMARY

Certain samples of potato tubers have been found to contain an interfering substance which is estimated as alcohol by the micro-method of Friedemann and Klass (1936) as modified by Saifi (1940). This non-alcohol material can be removed by washing the alcohol-distillate with purified heptane.

Both alcohol and the interfering non-alcohol material were shown to accumulate in potatoes held in nitrogen.

In earlier work (Saifi, 1940; Barker and Saifi, 1951) the micro-method of Friedemann and Klass (1936) was modified for use in estimating alcohol in potato tissue. The method finally adopted, for convenience of reference called method A, was as follows. The clearing agents (20 ml. 10 per cent. sodium tungstate and 20 ml. 10 per cent. mercuric sulphate dissolved in 2N sulphuric acid) were added to 20 g. potato tissue, as frozen powder, and the mixture left for 4 to 6 hours at room temperature with occasional shaking. This period allowed equilibrium to be attained between the alcohol content of the tissue and the solution. The alcohol was then distilled from an aliquot of the supernatant solution, the alcohol-distillate oxidized with alkaline permanganate, and the residual permanganate titrated with thiosulphate. The final oxidation and titration procedure were as described by Friedemann and Klass. The method is thus not specific; any volatile substance which is not retained by the clearing agents and which reduces alkaline permanganate would be included in the estimation. The term alcohol as used throughout the note does not, therefore, necessarily signify only ethanol.

Method A gave consistent results with the stocks of potatoes tested in 1939 and 1940 and the recovery of ethanol added to the tissue was 98·0±1·0 per cent. Later, however (i.e. in 1950), variable figures for alcohol content were obtained with the potatoes then in use, particularly with samples which had been held in nitrogen for about 25 days at 10° C. Moreover, re-distillation from lime and mercuric sulphate, as recommended by Friedemann and Klass for certain tissues, gave a distillate which appeared to contain more alcohol than the original distillate. Passing the distillate before condensation through a trap, containing saturated sodium hydroxide and mercuric chloride (Kozelka and Hine, 1941), gave similar results. This was evidence that, in addition to alcohol, some non-alcohol material was being oxidized by the alkaline permanganate.

The problem of removing the interfering material appears to have been

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solved by washing the distillate with purified heptane before the final oxidation. The figures obtained for the alcohol content of potato tissue were then reproducible and were not affected by re-distillation. The material which distils over and is oxidized by alkaline permanganate, following heptane washing, has been provisionally accepted as being ethanol; no specific test has been used, however, to show whether or not all this material is in fact ethanol. The recovery of ethanol after heptane washing was 98·0 per cent.

Method A was also modified in order to avoid as far as possible the use of strongly acid conditions during clearing and extraction. Thus Barker and Saifi (1951) had already shown that 'alcohol' is produced, presumably by splitting of methyl groups due to the strongly acid conditions, when pure pectin is added to the clearing reagents and the mixture distilled as in method A.

Method A, with heptane washing and the other modifications mentioned above, is called method B, the details of which are brought together in the following section. Using method B, the recovery of ethanol added to potato tissue was 96 per cent.

DETAILS OF METHOD B

To 20 g. of the sample of frozen potato powder add 20 ml. 10 per cent. sodium tungstate, 20 ml. 10 per cent. mercuric sulphate in 2N sulphuric acid, and 25 ml. distilled water. Shake for about 1 minute and add N.NaOH (about 40 ml.) to bring to pH 6·0 to 6·5. Allow to stand for 1 hour with occasional shaking, centrifuge until the supernatant solution is clear, filter, and make up to 200 ml.

An aliquot of the cleared solution is distilled through a trap containing 15 ml. saturated mercuric chloride and 15 ml. saturated sodium hydroxide (Kozelka and Hine, 1941). The distillate of about 40 ml. is washed in a separating funnel with 25 ml. of purified heptane (see below). The lower aqueous layer is then run off, water added to make up to 50 ml., and an aliquot taken for oxidation and titration as in Friedemann and Klass (1936).

The heptane was purified (in order to reduce its blank) by treatment with conc. sulphuric acid. 800 ml. of commercial heptane were shaken in a separating funnel with 75 ml. conc. sulphuric acid and the mixture left overnight. The upper layer of heptane was refluxed with 125 ml. 10 per cent. potassium permanganate and 10 ml. of 5N sodium hydroxide for 3–4 hours. The heptane was then distilled, the first 100 ml. and the last 200 ml. being discarded. The middle fraction was ready for use, after being well washed with distilled water.

RESULTS OBTAINED WITH METHOD B

Method B was used to follow the changes in alcohol content in a series of samples of immature potatoes, variety 'King Edward VII', held in nitrogen at −0·4° C. The alcohol increased progressively from the value of 0·45 mg. per 100 g. fresh weight in the initial air-sample to about 95 mg. after 14 days, column 3 of Table I; the content was maintained at about 98 mg. from 21 to 35 days, when observations ceased.

Omitting heptane washing gave the series of values for apparent alcohol presented in column 4 of Table I. Because of the very low content of alcohol in the air-sample, a non-heptane analysis was not made on this sample, Table I, but after only 2 days in nitrogen the figure for apparent alcohol (no heptane washing) exceeded that for alcohol (with heptane washing). After 14 and 35 days in nitrogen the value for apparent alcohol was more than twice that obtained with heptane washing.

No attempt was made to determine the amount of interfering material separately. If, however, the difference between the figures obtained for alcohol and apparent alcohol is assumed to be a direct measure of the amount of the interfering material, the values of column 5 of Table I show its magnitude expressed as mg. alcohol. On this basis, and neglecting the low value obtained after 28 days in nitrogen, the content of interfering material increased progressively in nitrogen and from 14 days onwards exceeded the amount of true alcohol. Moreover, it should be noted that an even higher value was obtained for the content of interfering material when the alcohol-distillate was oxygenated prior to oxidation with permanganate.

Further evidence is needed to show whether the interfering material is a product of fermentation or is formed in reactions not directly concerned with this process. The above results raise doubts, however, whether some of the alcohol/CO_2 quotients, previously reported for potatoes, may not be unduly high because of the inclusion of a proportion of non-alcohol material in the alcohol content as determined.

TABLE I

Changes in the contents of alcohol, of apparent alcohol, and of interfering material in immature potatoes held in air and in nitrogen at −0·4° C. (values expressed as mg. alcohol per 100 g. fresh weight)

1	2	3	4	5
Conditions	Days	Alcohol (with heptane washing)	Apparent alcohol (without heptane washing)	Interfering material (4–3)
In air	0	0·5	—	—
,, ,,	14	0·4	—	—
In nitrogen	1	0·5*	—	—
		3·0	—	—
,, ,,	2	6·7*	—	—
		9·8	13·8	4·0
,, ,,	4	23·0	—	—
,, ,,	7	44·8	—	—
,, ,,	10	62·0*	—	—
		55·0	—	—
,, ,,	11	69·1	131·0	61·9
,, ,,	14	96·8*	—	—
		92·0	224·0	132·0
,, ,,	21	98·8	—	—
,, ,,	28	98·0	121·0	23·0
,, ,,	35	98·8*	—	—
		98·5	238·0	139·5

* These are duplicate analyses of separate samples each of 25 potatoes.

ALCOHOL CONTENT OF POTATOES HELD IN AIR

Earlier evidence (Saifi, 1940; Barker and Saifi, 1951) suggested that low-sugar potato tubers, held in air at 10° C., might contain no free alcohol; thus, as noted above, the small amounts, ranging from 1·0 to 5·0 mg. per 100 g. fresh weight, estimated by method A might be produced from pectin or other material by splitting of methyl or ethyl groups during the extraction in the acid medium (pH 1·2). That the acid conditions of extraction can increase the apparent alcohol content of potato tissue was shown by using method B without neutralizing the acid clearing reagents (i.e. the potato powder was allowed to stand with the acid reagents for 1 hour prior to centrifuging without neutralizing). With this procedure a value of 3·4 mg. was obtained for the apparent alcohol content of the 0-day air-sample of Table I as compared with the value of 0·45 mg. as determined by the complete method B.

Using method B very low contents (0·0 to 0·5 mg. per 100 g. fresh weight) of alcohol were obtained in two other stocks of potatoes in addition to that which gave the results of Table I. We may thus conclude that little, if any, true free alcohol is present in certain stocks of potatoes held in air. In other stocks, however, even when the complete method B was used, values of about 5 mg. of apparent alcohol were obtained. Further investigation is needed to show whether this content actually represents true free alcohol or is due to interfering material.

I wish to thank Dr. F. A. Isherwood and Dr. L. W. Mapson for their help. The experimental work was carried out by Mr. A. W. Tomalin.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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Electron Microscope Observations on the Zoospores of Pylaiella and Laminaria

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WITH EIGHT PLATES

SUMMARY

The zoospores of *Laminaria saccharina* and *Pylaiella litoralis* have both been shown to possess a hairy front flagellum (Flimmergeissel). In both cases this is longer than the smooth hind flagellum. There is no proboscis. In *Pylaiella* it has been shown that the hairs are in two rows on the front flagellum and that they arise in pairs except at the extreme front end. Each hair has a jointed structure and consists of a thicker basal portion which passes abruptly into a thin distal portion. There are signs of a loose transparent skin covering the axis of the flagellum between the rows of hairs. The axis has also been shown to be fibrillar in construction and to be capable of decomposing into eleven strands, two of which are central. There is perhaps an intercalary material in the axis in addition to the component fibrils.

In a previous communication on the spermatozoid of *Fucus* (Manton and Clarke, 1950) such striking new facts had been revealed by the electron microscope as to make an extension of the inquiry to other types of motile cells in the Brown Algae a matter of immediate concern. The two most important points on which comparisons were required were the peculiar membranous proboscis on the *Fucus* male gamete and the hairy front flagellum (Flimmergeissel). Further information on both these organs from within the Fucaceae will be published elsewhere, but the present note adds some comparable facts for the asexual zoospores of two other members of the Brown Algae belonging to the Ectocarpaceae and Laminariaceae respectively.

Laminaria may perhaps be taken first although for convenience of presentation it is placed last in the series of illustrations. Figs. 14–17 show views of the zoospores of *Laminaria saccharina*. Plants of this species had been sent in from the Welsh coast for teaching purposes and had been stored in a refrigerator overnight. When examined next day by a class of undergraduate students, a vigorous liberation of spores was detected in a few pieces which were immediately isolated in fresh sea-water. Liberation continued for several minutes, during which time a number of mounts were made on prepared formvar carriers in the manner previously described for *Fucus*. The residual material, after killing with osmic vapour, was preserved in formalin diluted with sea-water. As in the case of *Fucus*, the preserved material was found to be at least as favourable as the living zoospores as a source of E.M. preparations, though neither was as easy to handle as the spermatozoids of *Fucus*. The zoospores of *Laminaria* are rather inconveniently large for electron microscopy and they become rather easily damaged. It also proved impossible to

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separate them from mucilaginous secretions liberated at the same time, which, after drying, form a granular or continuous deposit over the field which cannot subsequently be removed. The resulting pictures are in consequence less elegant than those obtained of the *Fucus* male gamete, although they give some important information.

As all these pictures (Figs. 14–17) reveal, there is no membranous proboscis. The two laterally attached (Fig. 16) flagella are unequal in length, the front one being considerably the longer (Figs. 14 and 17). This also is a difference from *Fucus* in which the front flagellum is considerably the shorter. This observation is, of course, not new, since algologists have long been aware of the somewhat anomalous condition characteristic of *Fucus*. In spite of this difference, however, the front flagellum in *Laminaria* as in *Fucus* is a Flimmergeissel. The hairs designated as 'Flimmer' are rather longer and less numerous in *Laminaria* than in *Fucus*, but essentially the front flagellum is the same organ in both, as can perhaps be seen most clearly in Fig. 17.

With the successful establishment of these facts, the study of *Laminaria* was discontinued in favour of *Pylaiella* for which a continuous supply of fresh material could be more easily obtained. As is well known, *Pylaiella litoralis* is a very common epiphyte on other seaweeds, notably on *Ascophyllum*, on which it can easily be dispatched by post and, if tightly wrapped and stored in a refrigerator, it will remain in good condition for a week. Unilocular sporangia are generally present throughout the winter months and they will liberate their zoospores very readily if the filaments are replaced in sea-water. Unlike *Fucus* and *Laminaria*, storage of the liberated cells in formalin after killing proved completely unsuccessful. The fresh spores, on the other hand, although possessing some of the disadvantages of *Laminaria* such as large size and ubiquitous secretions making dirty preparations, could be obtained in such abundance that ruthless discarding of imperfect mounts was no hardship.

Figs. 1 and 2 show two cells as seen with the electron microscope at the very low magnifications of ×3,000 and ×4,000 respectively. Figs. 3 and 4, which are added for comparison, represent similar cells in the fresh condition floating in sea-water as seen under the highest powers available to the light microscope. They are high-power ultraviolet photographs of two cells of unequal size (that of Fig. 3 is unusually small) taken with a Zeiss glycerine immersion objective working with the wave-length 2,750 Å at a magnification of ×3,000. As comparison with Fig. 1 indicates, the greatest detectable distortion in the electron micrographs produced by the drying of the specimen is a shrinkage in volume of the body; the lengths of the two flagella are little if at all altered. In spite of this shrinkage the same general features are displayed with varying degrees of clarity in all four pictures. A proboscis seems to be completely absent and of the two unequal flagella the long anterior one, as in *Laminaria*, is a Flimmergeissel. Further details about the structure of this flagellum are contained in the more highly magnified pictures which follow, but it is perhaps of interest to notice that although the hairs of the 'Flimmer' are not individually resolved by the ultraviolet microscope, their presence is nevertheless attested by a characteristic diffraction pattern detectable in the original prints of both Figs. 3 and 4 although more clearly contained in Fig. 3.

Further details about the very curious hairy appendages constituting the 'Flimmer' can be obtained from Figs. 5–9. Figs. 6 and 7 are enlarged portions of an unusually perfect specimen which is shown complete on a smaller scale in Fig. 5. The hairs are borne throughout its length except for the curiously contracted apical region (Figs. 5 and 6). Near the tip they are borne singly, but elsewhere they are in tufts of at least two together. This is very clearly detectable near the right-hand edge of Fig. 6 and also in parts of Figs. 7–9. These tufts are produced at fairly regular intervals and in two rows only, running longitudinally down the flagellum. This is extremely well shown by the somewhat decomposed flagellum illustrated in Fig. 9.

The structure of the individual hairs is more complex than previous information had led one to expect. As may be seen in Fig. 7 and less well in Figs. 6 and 8, the basal half of the hair is liable to be thicker than the distal part, the transition from one part to the other being abrupt and the exceedingly delicate apical region being often bent at an angle to the base which bears it. The significance of these observations is wholly unknown.

In addition to the hairs and the main axis from which they spring there are fairly definite signs of a delicate transparent skin round the latter which, in the dried condition, is somewhat loose owing possibly to shrinkage of the axis. This skin can be seen along one side of the base of the flagellum of Fig. 5, and on the upper side of the flagellum of Figs. 7 and 9, in both of which it unmistakably underlies the attachment of the hairs to the axis. In other cases, e.g. Fig. 8, it may become visible on both sides of the axis, on one of which it may cover and obscure the attachment of the hairs and on the other underlie them. It therefore seems probable that it is not simply a flat membrane but that it is a tube which flattens down in various positions on drying without being in any special way involved in the attachment of the 'Flimmer'.

Further information on other details of the construction of the flagellum can be obtained from the partially dismembered specimens illustrated in Figs. 10–13. Fig. 10 is from a cell in which the body has become torn off in the process of mounting. The hind flagellum is still recognizable as such to the left of the figure. The front flagellum in contrast shows almost complete fibrillar disintegration. A more highly magnified view of the front end is contained in Fig. 11. The 'Flimmer' is hard to detect, though traces of it still remain. The transparent skin has vanished, but the main flagellar axis has decomposed into eleven strands, two of which are central and rather closely coherent.

A similarly enlarged view of part of a somewhat better specimen is contained in Fig. 12. The remains of the two rows of 'Flimmer' litter the field on the two sides and are still partly attached to adjacent fibrils. The main axis has, however, again decomposed into eleven strands or, speaking perhaps more accurately, into nine strands and a central pair which, in the region shown, are still close enough together to give a ribbon-like appearance to the pair.

This type of fibrillar disintegration, though uncommon compared with the vast number of normal cells which have been examined, has been encountered sufficiently often to make it reasonably certain that the numerical details are significant. We have micrographs of at least six specimens in which the same numbers can be made out in their most fully dismembered portions. Fig. 13, on the other hand, shows an appearance which has been met with only once. Its interpretation is not completely certain, but it is inserted as the clearest evidence so far to hand indicating the possible presence of an intercalary substance in the interior of the flagellum. The longitudinal strands are imperfectly separated and the full eleven cannot be individually discerned although they are almost certainly present. The peculiar appearance is, however, caused by the horizontal strands spread across them like the rungs of a ladder. These seem to involve too much of the flagellum to be caused by the 'Flimmer', which is also to a limited extent still present. It is perhaps the remains of the disintegrating skin. It could equally be the remains of an intercalary material between the strands which in the other cases has been dispersed. Without other evidence the substance cannot be identified, but it is perhaps desirable to stress its existence even in this one specimen in order to discourage any tendency to over-simplify the evidence. A Flimmergeissel is undoubtedly a highly compound and complex organ and it may well contain a variety of structural components in addition to the more obvious parts which build up its gross morphology.

DISCUSSION

At this stage it is unnecessary to make more than a very few points by way of discussion. The absence of a proboscis on both these zoospores shows this organ to be confined, on our present evidence, to the spermatozoids of the Fucaceae. The Flimmergeissel, on the other hand, is common to the Fucaceae, Laminariaceae, and Ectocarpaceae and is therefore likely to be a fundamental character in the Brown Algae as a whole. Its construction has been shown to be considerably more complex than could be detected at the outset and much more information will be required before its behaviour can be elucidated. On the other hand, although very different in many ways from the cilia previously described in the higher plants (Manton and Clarke, 1951), an unexpected point of agreement has been revealed after fibrillar disintegration. In fern cilia also, which are quite without any trace of hairy appendages ('Flimmer'), a disintegration into eleven strands, two of which are different from the others and centrally placed, was found. It is premature to explore the possible meaning of this agreement, but it is perhaps of sufficient importance to deserve notice in passing.

ACKNOWLEDGEMENTS

This work has been carried out on the Cooke, Troughton, and Simms ultraviolet microscope installed in the Botany Department of Leeds University with the aid of a grant from the Royal Society and on the Philips electron microscope provided by means of a grant from the D.S.I.R.

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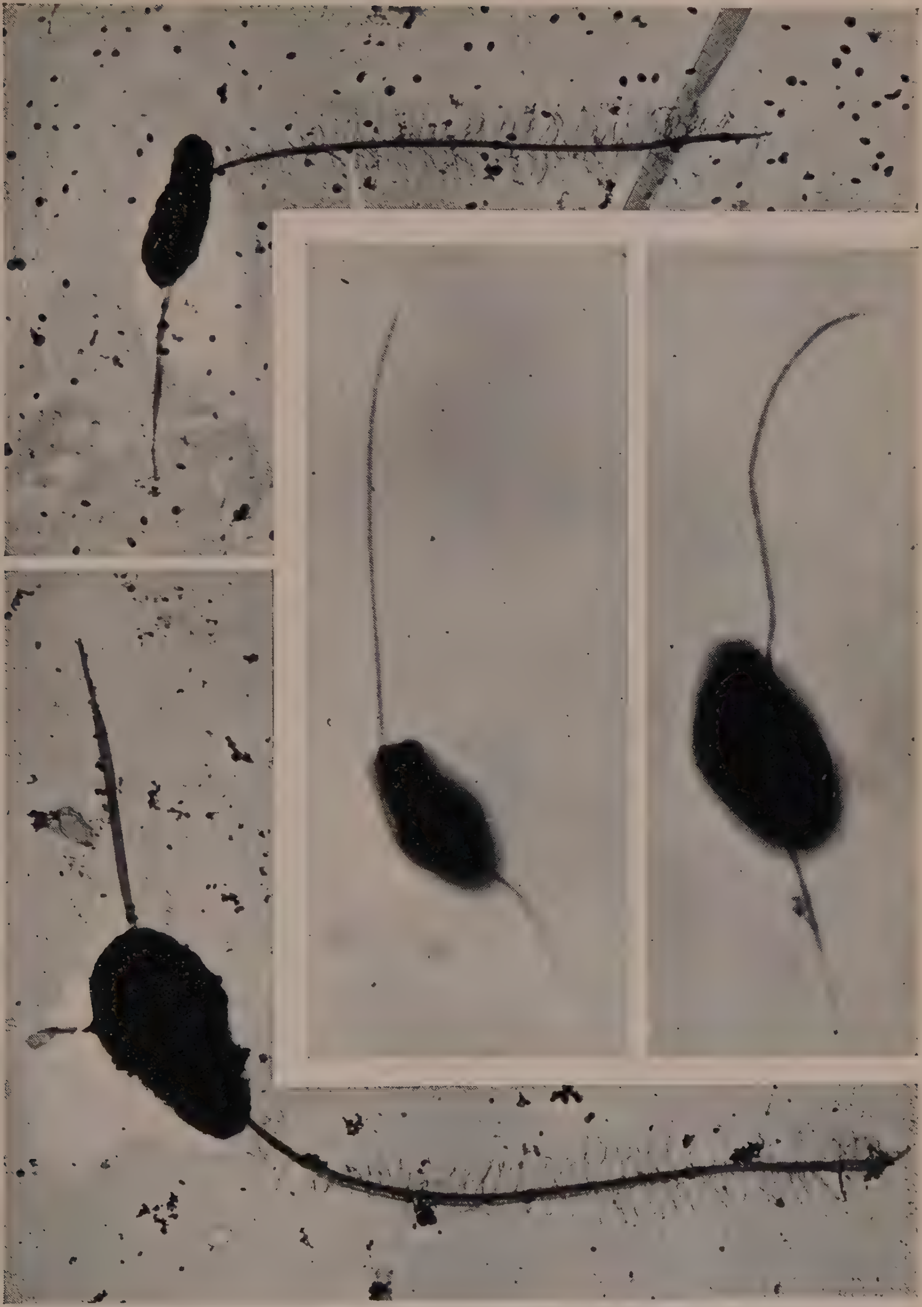
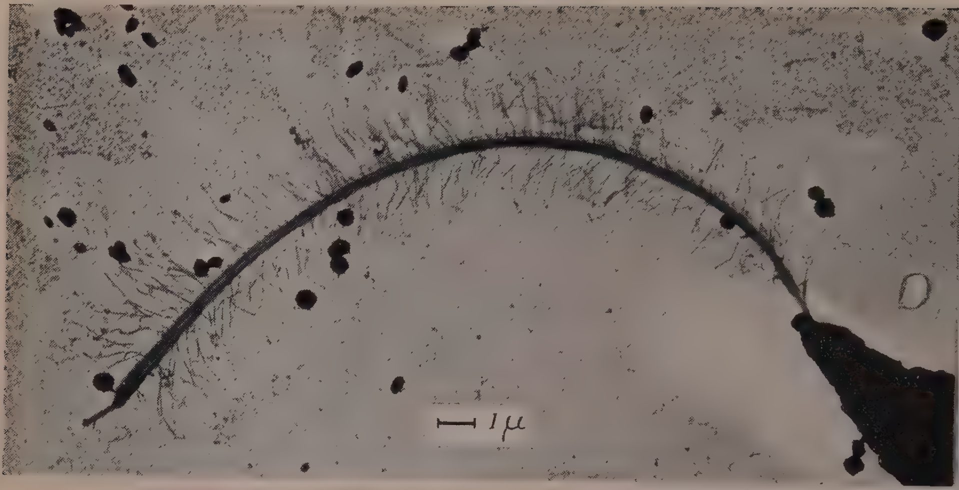


Fig. 1. Zoospore of *P. litoralis* killed on formvar carrier with osmic vapour. Electron micrograph M 39.31, gold-palladium shadowing, 60 kV., magnification ×3,000.

Fig. 2. Another cell of the same. Electron micrograph M 39.24, magnification ×4,000.

Figs. 3 and 4. Ultraviolet photographs (204.3b and 205.Ab), magnification ×3,000, of freshly killed zoospores of *Pylaiella litoralis* mounted in sea-water. Fig. 3 (on left) is an unusually small cell. The optical effects due to the presence of the hairs ('Flimmer') on the front flagellum are faintly visible.

I. MANTON AND B. CLARKE—PLATE I



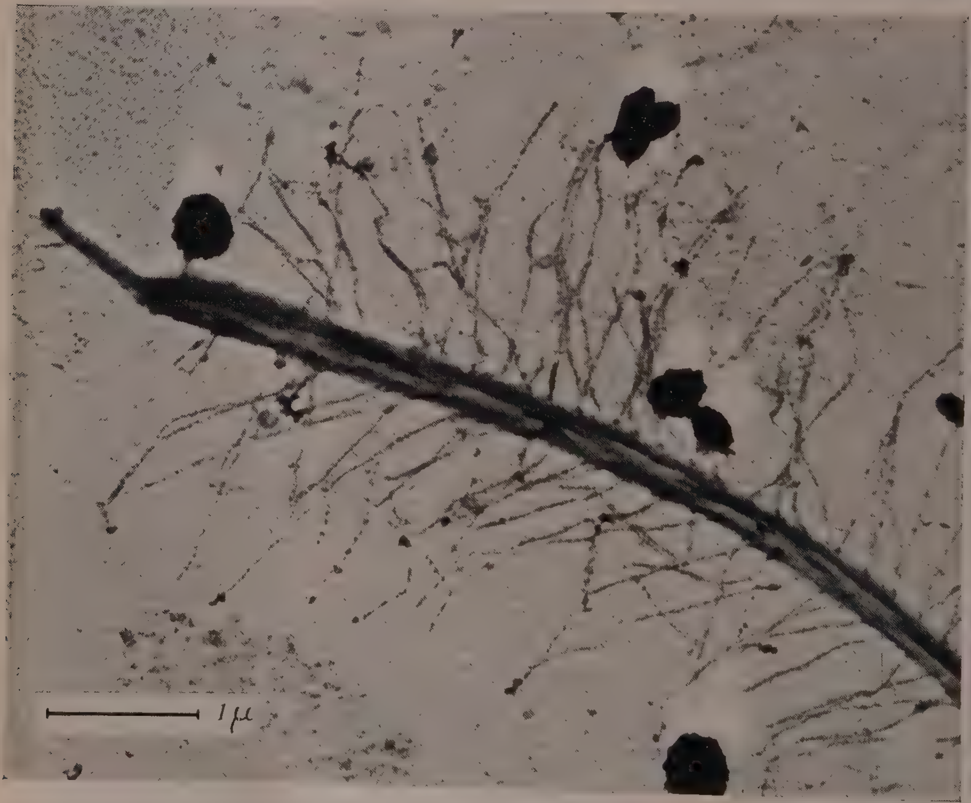
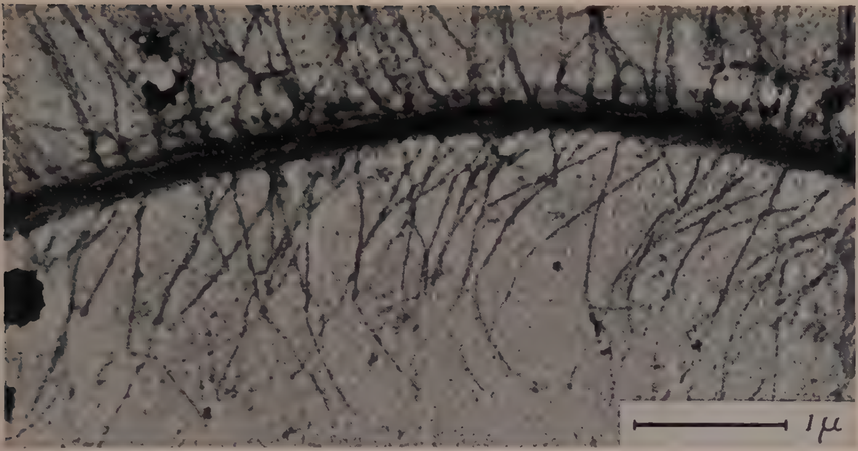


FIG. 5. *Pylaiella litoralis*, an exceptionally good front flagellum, other details as in Fig. 1. Electron micrograph M 39.1, magnification × 5,000.

FIG. 6. Enlarged detail of the front end of the flagellum of Fig. 5. Electron micrograph M 39.4, magnification × 20,000.

I. MANTON AND B. CLARKE—PLATE II



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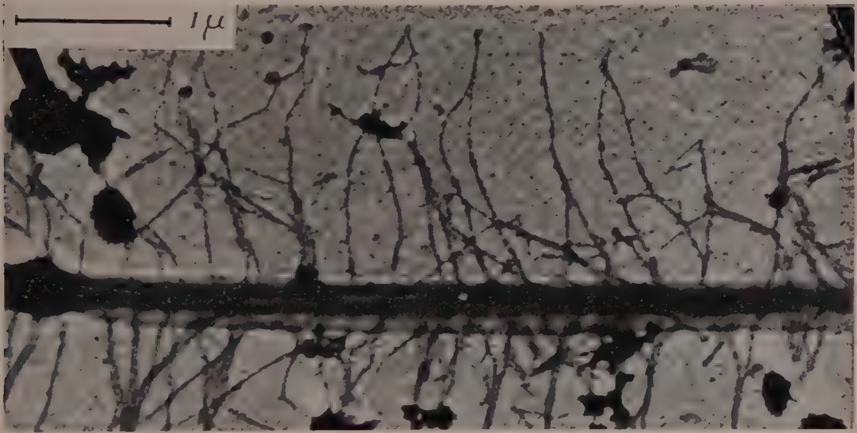

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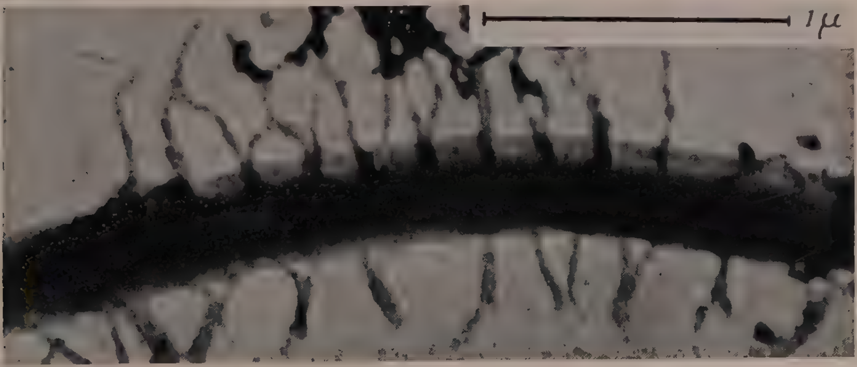

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FIG. 7. Enlarged detail of the middle portion of the flagellum of Fig. 5 showing jointed hairs. Electron micrograph M 39.5, magnification ×20,000.

FIG. 8. Enlarged detail of part of the flagellum of Fig. 1 showing the skin overlying the bases of the hairs on the lower side. Electron micrograph M 39.30, magnification ×20,000.

FIG. 9. Still more enlarged detail of a partly decomposed flagellum showing the mode of origin of the bases of the hairs in tufts and in two rows. Electron micrograph M 30.15, uranium shadowing, 40 kV., magnification ×40,000.

I. MANTON AND B. CLARKE—PLATE III

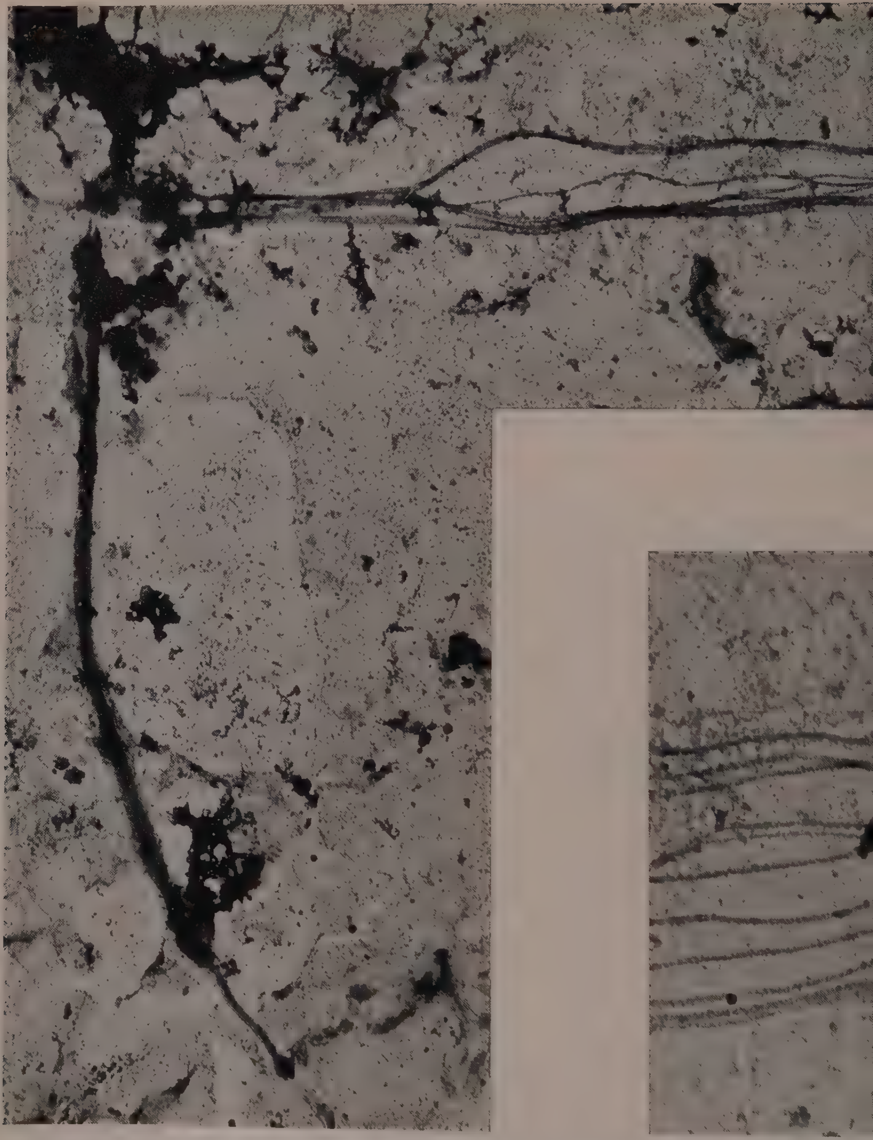
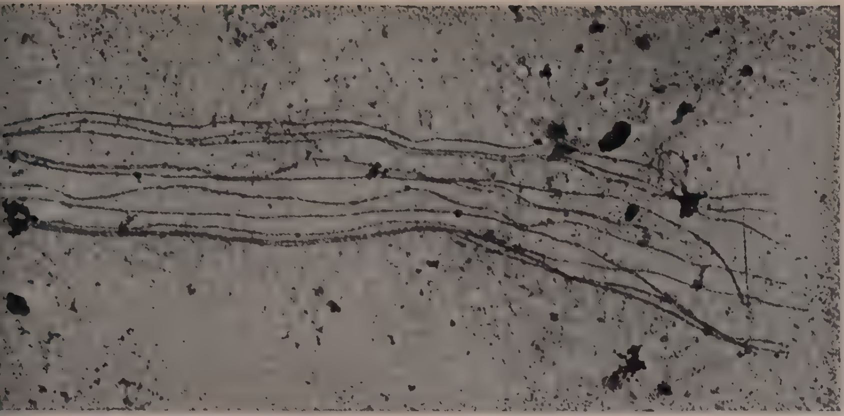


FIG. 10. *Pylaiella litoralis*, much decomposed cell with the body torn away. The micrographs M 39.17 and 18, chromium shadowing, magnification × 10,000.

FIG. 11. Part of the same, more highly magnified, showing eleven component

I. MANTON



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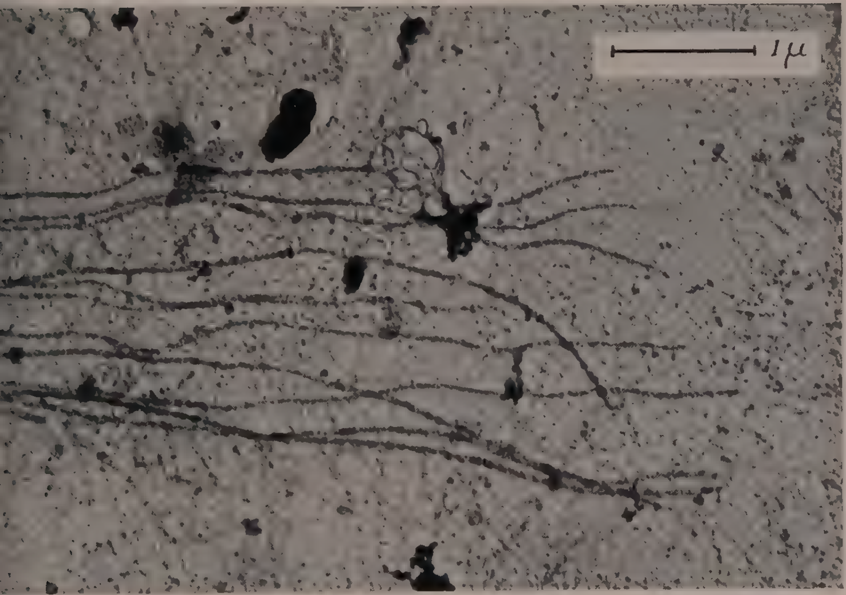
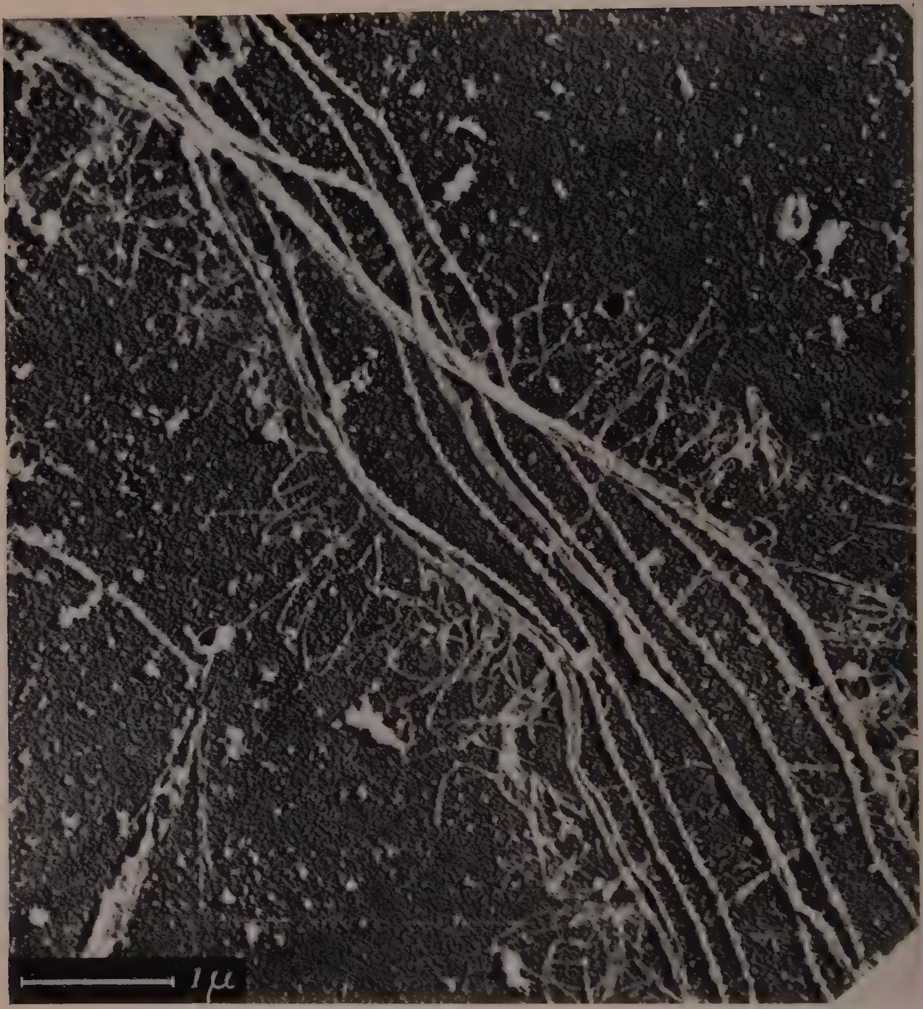


11

m still intact but the long front flagellum showing fibrillar disintegration. Electron

micrograph M 39.20, magnification × about 19,000.

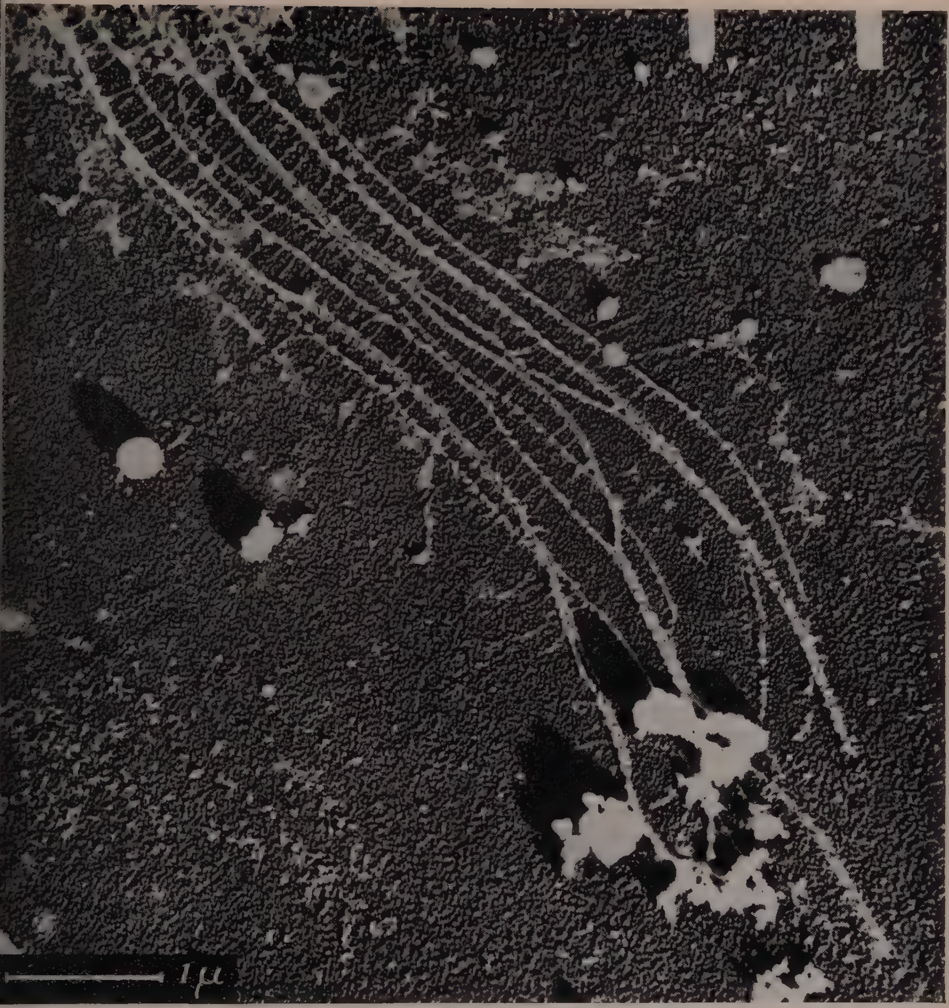
KE—PLATE IV



12

FIG. 12. *Pylaiella litoralis*. Part of a front flagellum in a state of fibrillar disintegration from a better specimen than the preceding. Electron micrograph M 39.8, gold-palladium shadowing, reversed print, magnification ×20,000.

I. MANTON AND B. CLARKE—PLATE V

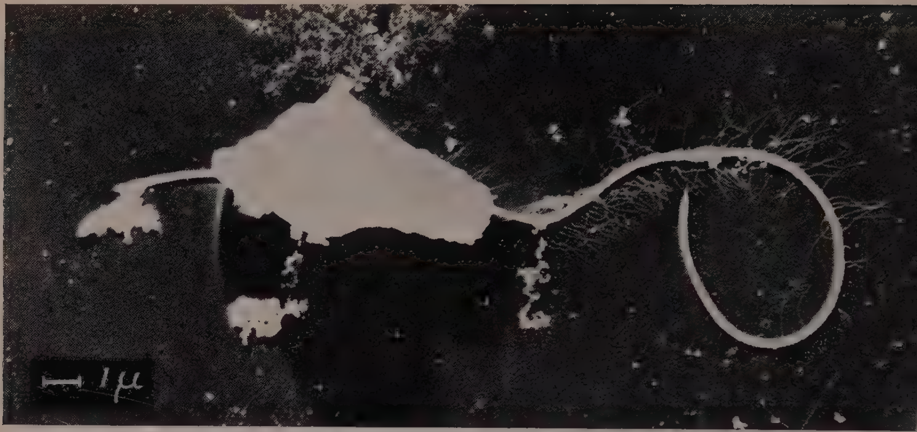


13

FIG. 13. *Pylaiella litoralis.* Part of another specimen showing incomplete fibrillar disintegration with traces of connecting substance between the strands. Electron micrograph M 36.33, chromium shadowing, reversed print, magnification ×20,000.

I. MANTON AND B. CLARKE—PLATE VI

14



15

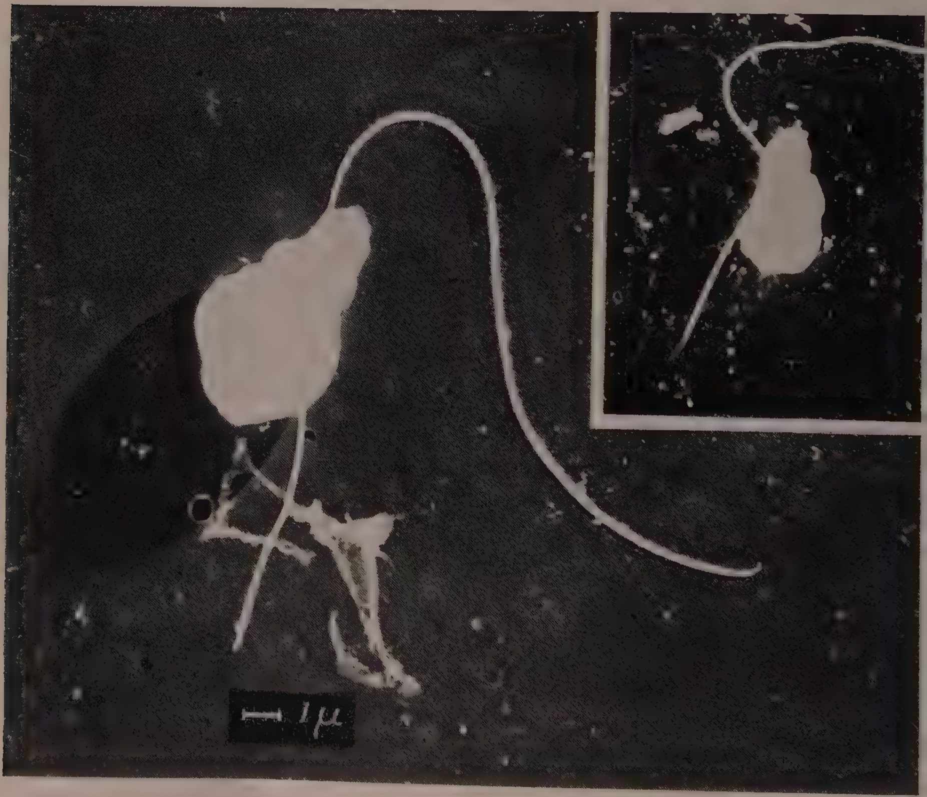
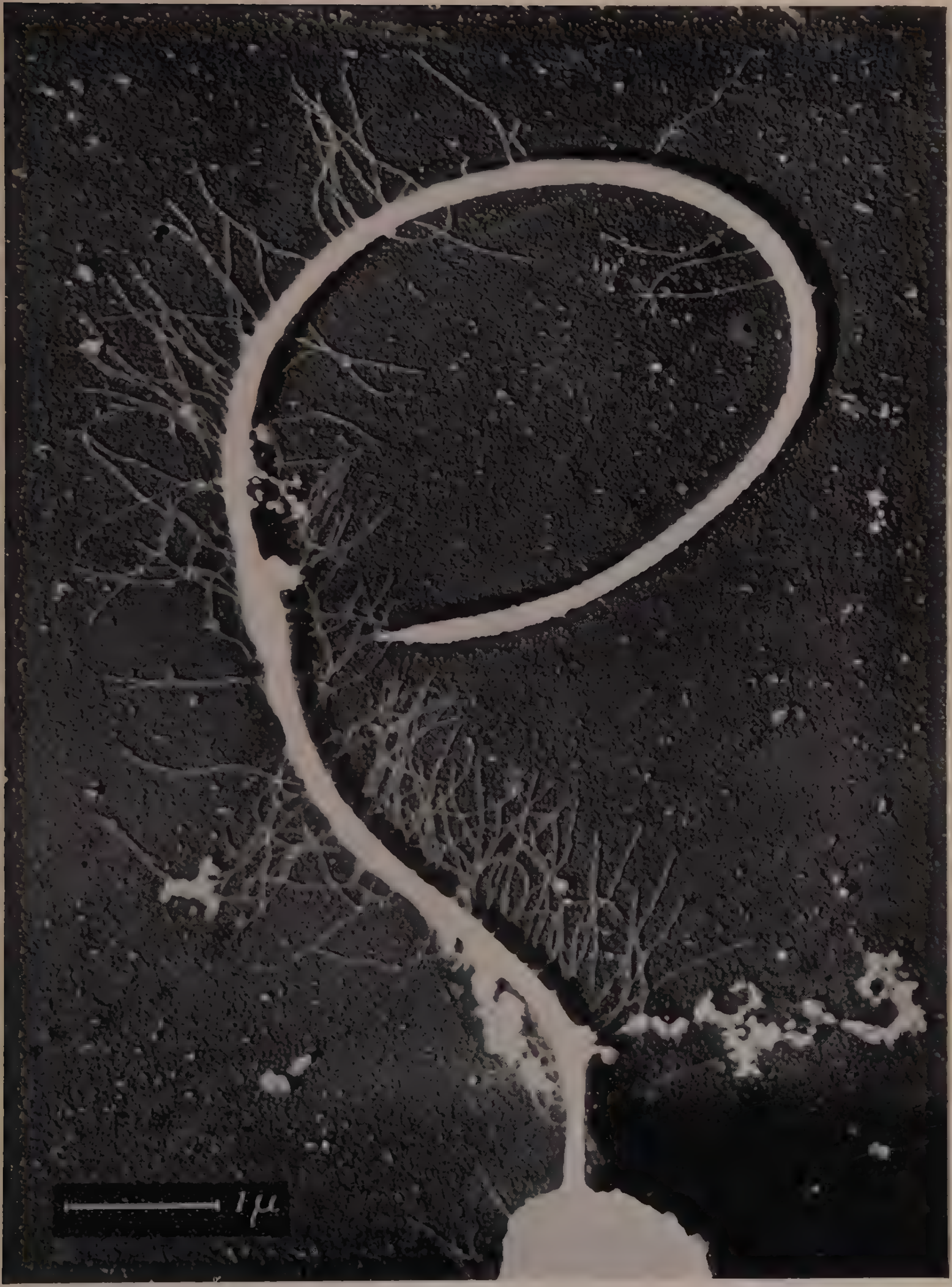


FIG. 14. *Laminaria saccharina* zoospore, material preserved in formalin after killing with osmic vapour. Electron micrograph M 32.14, uranium shadowing, 40 kV., magnification × 5,000.

FIG. 15. Another cell otherwise similar to Fig. 14. Electron micrograph M 31.2, magnification × 5,000.

FIG. 16. Another cell, killed on carrier without formalin treatment, otherwise similar to Fig. 14. Electron micrograph M 32.5, at 60 kV., magnification × 2,500.

I. MANTON AND B. CLARKE—PLATE VII



17

FIG. 17. Detail of the front flagellum of the cell of Fig. 14. Electron micrograph M 32.16, at 60 kV., magnification × 20,000.

I. MANTON AND B. CLARKE—PLATE VIII

Electron Microscopic Investigations of the Walls of Green Algae

I. A PRELIMINARY ACCOUNT OF WALL LAMELLATION AND DEPOSITION IN *VALONIA VENTRICOSA*

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Biophysics Section, Botany Department, University of Leeds

WITH SEVEN PLATES

SUMMARY

A survey of the structure of wall lamellae in the green alga *Valonia ventricosa* has revealed the following details. The microfibrils are not so constant in width as had previously been suggested; they vary in this dimension from about 100 Å to about 350 A. The length of the shadow cast by separated microfibrils shadowed with palladium-gold or with uranium suggests that the microfibrils are flattish ribbons rather than circular cylinders. In the intermediate lamellae of the wall they are in intimate contact, probably with an interfibrillar cementing material, but in outer lamellae they become more or less widely separated. This separation is assumed to occur as a result of adjustment to size increase in the vesicle. Observation of the innermost lamella shows that the microfibrils here are arranged at random, in marked contrast to the condition in other lamellae. Here and there granular deposits on this lamella appear to be connected with the cytoplasm, and it is suggested that these may represent points in the cytoplasm associated with microfibril production. Occasional twisting of microfibrils over and around each other in most of the wall lamellae suggests most strongly that the wall and the cytoplasm are not so clearly separated during deposition as has been thought.

INTRODUCTION

It is now some fourteen years since the crossed fibrillar structure was first revealed in the walls of the alga *Valonia* (Preston and Astbury, 1937). The structure was at that time deduced from X-ray diffraction patterns and, though since that time little further work has been done on *Valonia* itself, the same type of structure has been found, again by X-ray methods, in *Chaetomorpha* (Nicolai and Frey-Wyssling, 1938), *Cladophora prolifera*, *Cl. rupestris*, and *Cl. gracilis* (Astbury and Preston, 1940), and, indeed, in all members of the Cladophoraceae except in the Spongomorpha group of *Cladophora* and in some other algae (Preston and Nicolai, 1951). Such a structure as this, in which the wall lamellae show an organization which varies periodically throughout the wall thickness, points most clearly to an orienting mechanism in the cytoplasm–wall interface; and up to 1948 the wall was considered as originating in this interface in such a way that the newest wall lamella could be pictured as a rather uniform sheet of cellulose lying immediately outside the cytoplasmic surface. In growing cells the mental picture was rather different, since it was known that the cytoplasm is more closely associated with the primary wall than it is with the secondary wall (Frey-Wyssling, 1948; Preston and Wardrop, 1949).

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[1] On leave from Philips Electrical, Ltd., Eindhoven, Holland.

In 1948, however, there appeared the first electron micrograph of an intact, untreated cell-wall, that of *Valonia ventricosa* (Preston *et al.*, 1948). This confirmed completely the crossed fibrillar structure. Further than this, however, the photographs revealed what had not until then been suspected, that the cellulose is organized into microfibrils whose diameter appeared to be rather uniform at about 250 Å and apparently endless. Confirmation soon followed that walls of higher plants are also built up of similar microfibrils (Frey-Wyssling *et al.*, 1948; Frey-Wyssling and Mühlethaler, 1949, 1950; Mühlethaler, 1949, 1950), though in conifer tracheides their diameter is reported to be less than the figure quoted for *Valonia* (Hodge and Wardrop, 1950). In *Valonia*, and in the other secondary walls examined up to this time, it appeared probable that the microfibrils run parallel to each other within any lamella of constant orientation. This contrasts sharply with the condition in primary walls, where they are clearly twisted around each other (Frey-Wyssling *et al.*, 1948; Mühlethaler, 1950), a difference which could be associated with a different cytoplasm–wall relationship. Apart from a general impression that they are cylindrical, the electron micrographs published so far give no obvious clue as to the shape of the microfibrils in cross-section.

More recently we have found at Leeds that the cellulose in the walls of a number of algae is constituted similarly to that in *Valonia* (Preston and Nicolai, 1951; Nicolai, 1951) and therefore these electron micrographs collectively suggest most strongly that cellulose in the native form is normally associated into microfibrils. Regenerated cellulose, on the other hand, whether produced artificially (Frey-Wyssling *et al.*, 1949) or in nature (Nicolai, 1951) does not show such markedly fibrillar structure, so that the microfibrils are apparently of biological rather than crystallographic origin.

During the same period the possibility has been demonstrated by electron micrography and otherwise, of breaking up cellulosic structures into particles some 600 Å long and 50–100 Å in diameter, recalling the classical micelles of Nägeli (Svedberg, 1949; Ranby, 1949; Ranby and Ribi, 1950). It seems probable therefore that the microfibrils may not be in any strict sense the fundamental units of structure, but this should not be allowed to detract from their biological significance.

Among the outstanding questions regarding these microfibrillar systems the following may therefore be listed as demanding urgent attention.

1. Are the microfibrils so uniform in diameter as these first electron micrographs seem to suggest?

2. Can the microfibrils of celluloses from all sources be hydrolysed into the units of structure observed by Ranby, or is it significant that in one of the two celluloses used by him (wood cellulose) the microfibrils themselves have been reported to be 100 Å in diameter or less? If this hydrolysis can be per-

formed, then the question as to the relation between these particles and the microfibrils becomes urgent.

3. What is the shape of the microfibrils in cross-section? This might be a particularly important question since the earlier X-ray work (Preston and Astbury, 1937) had shown that the planes of 6·1 Å spacing in the *Valonia* wall tend to lie parallel to the wall surface. This means that the 6·1 planes must lie parallel within each microfibril and the microfibrils are not oriented at random about their length. If the microfibrils are cylindrical, then this uniplanar orientation must arise through a non-uniform distribution of groupings—probably —OH groups—on the microfibril surface.

4. Is the distinction between 'parallel' (i.e. not twisted) microfibrils in secondary walls and the 'twisted' microfibrils in primary walls a valid one? This can be answered only by the most careful observation of material subjected to a minimum of handling.

5. Can anything be seen of the relationship between the microfibrils and the cytoplasm? An attempt to answer this would need observation of the innermost wall lamella as nearly intact as possible and with some adhering cytoplasm. Some observations have already been made along these lines, but they have involved either dissection of higher plant material in a blendor (Mühlethaler, 1950) or the use of walls which have to be subjected to drastic chemical treatment which would not be expected to leave other than mere traces of the cytoplasm (Roelefsen, 1951). In neither case was any observation made on the relation between the wall and the cytoplasm. Obviously it would be better to use a larger cell than those observed by these investigators, which would allow the observational material to be identified without the ambiguity consequent upon dissection in a blendor.

During the past few months the very suitable features of *Valonia* have been exploited in an attempt to answer these questions. The wall is so finely lamellated that, using a stripping technique similar to that employed by Reed and Rudall (1948) on worm cuticles, all parts of the wall are readily observable; the wall is very robust and large pieces of any lamella can be obtained without drastic treatment and its position in the wall identified; and large quantities of microfibrils can be obtained from one and the same cell, thus obviating any interspecies or even inter-individual variance.

The main concern of this first paper is with question 5 listed above—the relation between the wall and the cytoplasm—and little account will be given here of what are as yet provisional answers to the other questions. Perhaps, however, since the electron micrographs presented here do contain answers to some of these other questions, the following points may properly be made at this time. In *Valonia* the microfibrils are not so uniform in diameter as the first electron micrographs suggested. Although on the whole they are predominantly about 250 Å wide, individual fibrils vary from about 100 Å to about 350 Å,[1] with a mean value somewhere about 200 Å. The present indications are that the diameter may be a multiple of about 80–100 Å, but this is as yet far from certain. The narrowness of the shadow cast on a film by isolated microfibrils shows that, under the conditions of observation, they are flat ribbons and not circular cylinders, the thickness to width ratio lying somewhere between $\frac{1}{3}$ and $\frac{1}{7}$. These features of the microfibrils and their degenerative breakdown will be discussed at length elsewhere.

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[1] The same point has been made by Wilson (*Ann. Bot.* 1951) in a publication issued while this paper was in the press.

MATERIALS AND METHODS

The only material available for this investigation consisted of a relatively large assembly of vesicles of *Valonia ventricosa* collected some years ago off the Dry Tortugas by Dr. (now Professor) F. C. Steward and presented at that time to the senior author. This was, therefore, the same batch of material, samples of which were used for the earlier X-ray examination. It is unfortunate that the investigation had to depend on preserved specimens, and the picture presented of the cytoplasm–wall relationship must remain provisional until living material is available.[1] Nevertheless the observations are so striking that they are presented here even at the risk of some error. The comparison photographs of the outer surface of the wall and of intermediate lamellae are presumably not subject to modification on preservation, and can therefore be taken as reliable.

In preparation for insertion in the electron microscope the material was treated as follows. A vesicle was washed quickly in distilled water, punctured, and the contents pressed out. Without any further washing the vesicle was then inflated with air and dried quickly. Pieces of wall a few millimetres square were then cut from the flatter parts of the wall and lamellae stripped off with Sellotape until only the lamella required for observation remained. This could then be mounted on the microscope grid without any supporting film, shadowed with palladium-gold or uranium, and examined directly. The technique was such as to ensure that no part of the lamella chosen came into contact with the Sellotape, since this produces gross artefacts.

Lamellae from three locations in the wall—outer surface, inner surface, and intermediate—are found to be so very different that they may be considered in detail separately.

The outer surface of the wall

Although the structure of the outermost lamella in the wall is rather variable, it seems rather clear that the electron micrographs presented in the earlier paper (Preston *et al.*, 1948) were of this surface. This is evidenced by Pl. I, Figs. 1 and 2, which represent one type of structure found. The crossed fibrillar structure is very much in evidence, but the uppermost lamella has become either very much torn (Pl. I, Fig. 1) or even disintegrated (Pl. I,

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[1] Fresh material, collected and freeze-dried at Naples, has now been shown to present essentially the same features.

Fig. 2). The two small patches on the upper left of Pl. I, Fig. 1, clearly suggest a tearing of outer lamellae during growth much as foreshadowed many years ago (Preston, 1931). Another type of distortion is presented in Pl. II, Fig. 1, where several underlying lamellae seem also to have undergone severe displacements. It may be significant that such torn or distorted lamellae are found particularly around large vesicles. In these photographs the outer surface only of the outer lamella is shadowed with metal; shadowing of the inner surface gives essentially the same picture and this is not reproduced here. All the photographs of this type show distinct microfibrils clearly separated. The smallest microfibrils are about 100 Å in width.

In some vesicles, however, and particularly with the smaller ones, large parts of the surface are covered by a very smooth, almost structureless lamella; so lacking in detail that there is no point in presenting any illustration here. This would seem to represent a cuticle which is apparently sloughed off as the vesicle enlarges.

Intermediate lamellae

Intermediate lamellae appear much more uniform. Normally whole lamellae can be stripped off free from any signs of an underlying lamella with a different fibrillar direction. The fibrils, too, are much more uniformly arranged, sometimes so closely packed (or cemented together) that the lamina appears almost smooth (Pl. II, Fig. 2). Two further common features are the presence of lenticular openings, ranging in width up to about 2,000 Å, and heavy granular deposits (Pl. III, Fig. 1). It is naturally not certain whether these lenticular openings are artefacts produced either during specimen preparation or in the electron beam. They may be seen immediately the beam is switched on and do not spread even at a beam intensity much higher than that normally used, so that it seems rather certain that these are integral parts of these lamellae. Similarly the granular deposits, which do not appear on other material processed in the same way, probably belong to the *Valonia* itself and not to any external contaminating source.

One further observation can be made with these lamellae, giving a most striking answer to the questions concerning the difference between secondary and primary wall layers. The general arrangement of the microfibrils here is so regular that occasional irregularities can readily be detected, and one such irregularity is an occasional twisting of one microfibril around its neighbour or even around one normally separated from it by many intervening microfibrils. Such twists may be observed at the points marked 'T' in Pl. III, Fig. 1, and it is quite clear that such effects could not conceivably have been induced by any treatment of the specimen. These are certainly a real feature of the wall. Observations such as these make it seem rather doubtful whether the conception of the deposition of secondary layers at a cytoplasm-wall interface can any longer be retained in any strict sense. This point will be discussed later.

The innermost lamella

By far the most interesting observations come, however, from the innermost lamellae, abutting on the cytoplasm. In attempting any interpretation of these it must be stressed that, as mentioned earlier, the material had been preserved in formalin for a number of years[1] and, further, that the conceptions involved are based on a series of photographs taken at different places on the wall surface with no spatial or temporal sequence. The following interpretation is therefore quite tentative and, in parts, speculative, though the actual observations themselves stand, of course, on their own merits.

The general impression is of a hazy, structureless flocculum overlying what is evidently a fibrous wall lamella much like those illustrated above (Pl. IV, Fig. 1). This is the type of observation made by Mühlethaler (1950) on maize coleoptiles. When this deposit is more or less intact, the glimpses which may be had of the underlying cellulose lamella show that the microfibrils are arranged, however, at random, very unlike the carefully ordered arrangement of the older lamellae (Pl. IV, Fig. 1). The lack of order in this innermost lamella is indeed a feature of all the photographs of this lamella which we have observed. When it is stripped off completely, the normal crossed fibrillar structure once more becomes manifest in the underlying lamellae (Pl. IV, Fig. 2).

Normally, i.e. over the large bulk of the surface of the lamella, there is no sign of any clear relationship between the cellulose microfibrils and this hazy, superposed structure which, like Mühlethaler (1950), we consider to be the remnants of the cytoplasm. Here and there, however, dense accumulations of this cytoplasmic material seem to adhere more firmly to the wall, since they still remain after the large bulk of the flocculent material has been removed; and at these points it is rather clear that the microfibrils are in association with these masses and indeed give the impression of arising from them (Pl. V, Fig. 1; Pl. VI, Fig. 1). Occasionally the cytoplasmic material is distinctly granular (with granules of diameter about 500 Å), and in the illustration presented here (Pl. VII, Fig. 1) its connexion with the microfibrils is most convincing.

DISCUSSION

Although it is the purpose of this communication to put the present observations on record rather than to make any lengthy discussion of them, there are nevertheless a few points which may well be taken up at this time.

Remembering that the lamellae referred to here as 'intermediate' make up the large bulk of the wall, the impression which is given by the present series of observations on the *Valonia* wall is one of much more close coherence than had been foreshadowed by the earlier electron micrographs. Although the wall is certainly microfibrillar in organization, these units of wall structure are very closely packed and are probably cemented together by a structureless interfibrillar cementing material. Such a structure harmonizes much better with the known impermeability of the *Valonia* wall than did the earlier conception of a loose 'weave' of separated microfibrils.

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[1] See footnote, p. 250.

This beautiful regularity of structure in the intermediate lamellae makes it equally certain that in the outer regions of the wall, from which lamellae were stripped, following identically the same technique as used with the intermediate, there has been considerable distortion during growth. Adjustments to dimensional changes are therefore made here by what appears to be mechanical separation of microfibrils, or groups of microfibrils, without any signs of the intervention of metabolic effects such as are known to occur in growing walls. The interfibrillar cementing material, which can be seen frequently in teased-out intermediate lamellae but rather seldom with these outer ones, is possibly digested in these outer regions, facilitating the mechanical separation of the microfibrils.

On the other hand, it seems fairly safe to conclude that the innermost lamella at least of the wall is penetrated by cytoplasm so that adjustments to dimension changes may for a time, i.e. until any particular lamella is buried sufficiently deeply in the wall, be metabolic rather than mechanical. The evidence for this is of two kinds. Firstly, since the microfibrils are twisted around each other, in the intermediate lamellae, in a way which could not have been induced during the preparation of the lamellae, then it is clear that there can have been no complete cleavage between wall and cytoplasm such as is commonly envisaged in the idea of deposition at a cytoplasm–wall interface. Secondly, examination of the innermost lamella itself has revealed 'islands' of what appear to be cytoplasmic material in close association with the cellulose microfibrils. An alternative interpretation of observations of this latter type could be that, if the microfibrils just deposited by the cytoplasm are more loosely attached to each other and to the older lamella than are those in these older lamellae themselves, then the whole appearance of the innermost lamella could be due to distortions during stripping and accumulation of fibrillar material here and there into heaps. This, however, seems highly unlikely on several grounds. The 'islands' referred to as cytoplasmic are clearly granular, not fibrillar; the wall covered with an intact layer of cytoplasm still allows the observation of disordered microfibrils much as they appear when this layer has gone; and the lamellae on which these observations have been made had never touched the stripping tape—the rest of the wall had been stripped from it. On these grounds we prefer the former interpretation.

The idea of localization of phenomena at the surface of the cytoplasm is, in any case, not new in cell physiology. Something of the same kind has frequently been invoked to explain phenomena associated with permeability properties, with enzyme systems, and so on. Nor, indeed, is the idea new in questions of wall deposition, for evidence of very different kinds has already suggested this idea on at least two occasions (Frey-Wyssling, 1948; Preston, 1951). This is, however, the first time that visual evidence has been put forward and, though confirmation must be awaited on fresh material,[1] there seems little room for doubt. One puzzling feature still remains. There can be no doubt but that, in the material used here, the microfibrils of the innermost lamella are arranged at random. One has to assume that these cells, or some of them at least, were still developing at the time of collection, so that this innermost layer would eventually have taken its place among the intermediate lamellae, as new material was deposited from the inside. The question therefore arises as to how these randomly arranged microfibrils come to assume the beautifully regular alinement which they later adopt. As yet we have no clue, but if the interpretations made here are correct, then it would seem that the formation of the microfibrils and their orientation are brought about by mechanisms which are separated in time and possibly in space.

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[1] See footnote, p. 250.

ACKNOWLEDGEMENTS

The senior author is indebted to the Department of Scientific and Industrial Research for a Grant provided to finance a research programme of which the results presented in this paper form a part.

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DESCRIPTION OF PLATES

All these electron micrographs are of wall lamellae in *Valonia* (not replicas), metal shadowed and without supporting film.

PLATE I

FIG. 1. Outermost lamella, uranium shadowed. Magnification 15,000 ×. Notice that the fibrils are separated in bands.

FIG. 2. As in Fig. 1, but magnification 25,000 ×. The outermost lamella is dissected almost into individual microfibrils.

PLATE II

FIG. 1. Outermost lamellae, palladium-gold shadowed, magnification 15,000 ×. Note the extreme distortion in all visible lamellae.

FIG. 2. Intermediate lamella, uranium shadowed, magnification 15,000 ×. Note the compactness of the lamella, giving an impression almost of smoothness.

PLATE III

FIG. 1. Intermediate lamella, palladium-gold shadowed, magnification 30,000 ×. Notice again the closeness of packing of the microfibrils and the regularity of their arrangement. Notice also (*a*) the two lenticular openings (see text), (*b*) the copious granules, (*c*) the 'twisting' of microfibrils at points marked T.

PLATE IV

FIG. 1. The innermost lamella with a covering of cytoplasm; palladium-gold shadowed, magnification 25,000 ×. Notice that below the flocculent cytoplasmic material the microfibrils are arranged at random.

FIG. 2. The innermost lamella with the cytoplasm and the randomly arranged microfibrils swept away (the white area to the lower right shows the limit of the cytoplasm). Note that in these lamellae, immediately below that being deposited at the time of fixation, the 'crossed fibrillar' texture is already manifest. Palladium-gold shadowed, magnification 13,000 ×.

PLATE V

FIG. 1. An aggregation of cytoplasmic material with apparent connexion with the randomly arranged microfibrils of the innermost lamella. Palladium-gold shadowed, magnification 15,000 ×.

PLATE VI

FIG. 1. A second example of the type of wall–cytoplasm relation illustrated in Pl. V, Fig. 1. Palladium-gold shadowed, magnification 15,000 ×.

PLATE VII

FIG. 1. As in Pl. V, Fig. 1, magnification 22,500 ×. Notice that here the cytoplasmic deposit is clearly granular, and observe the way in which many groups of microfibrils turn into this deposit.

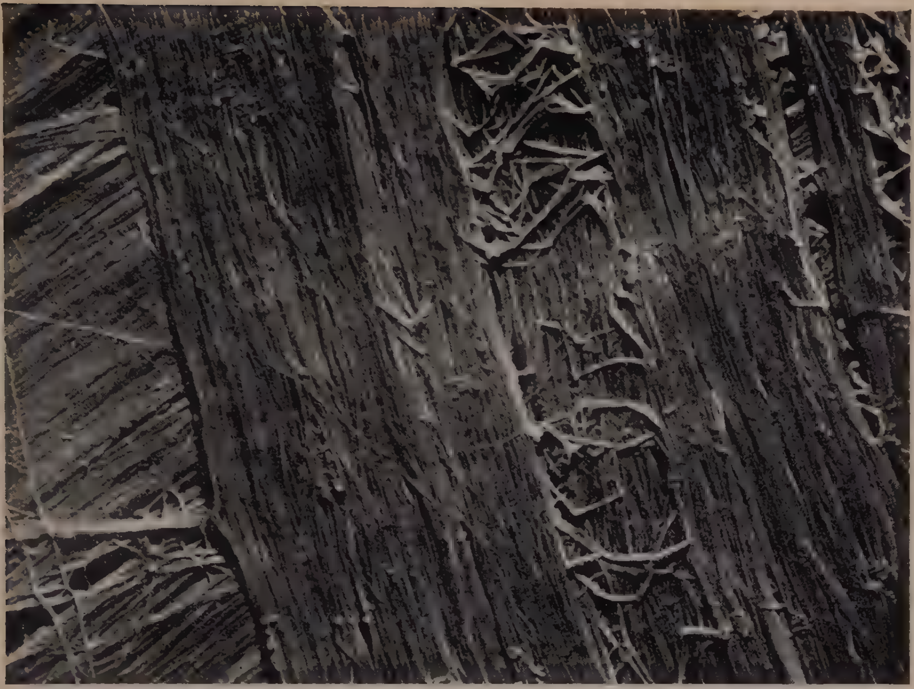


FIG. 1

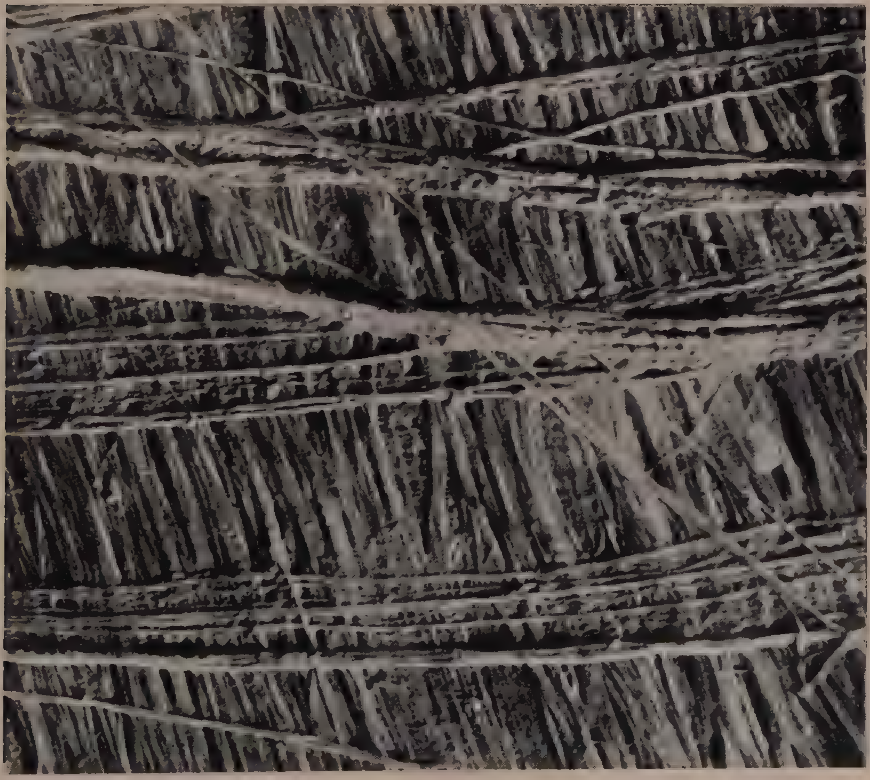


FIG. 2

R. D. PRESTON AND B. KUYPER—PLATE I

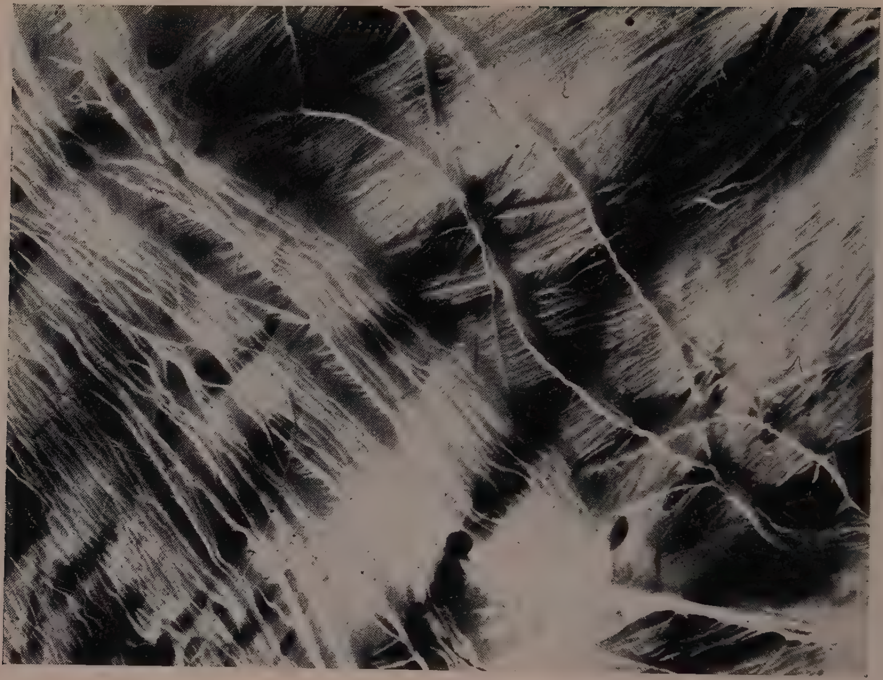


FIG. 1

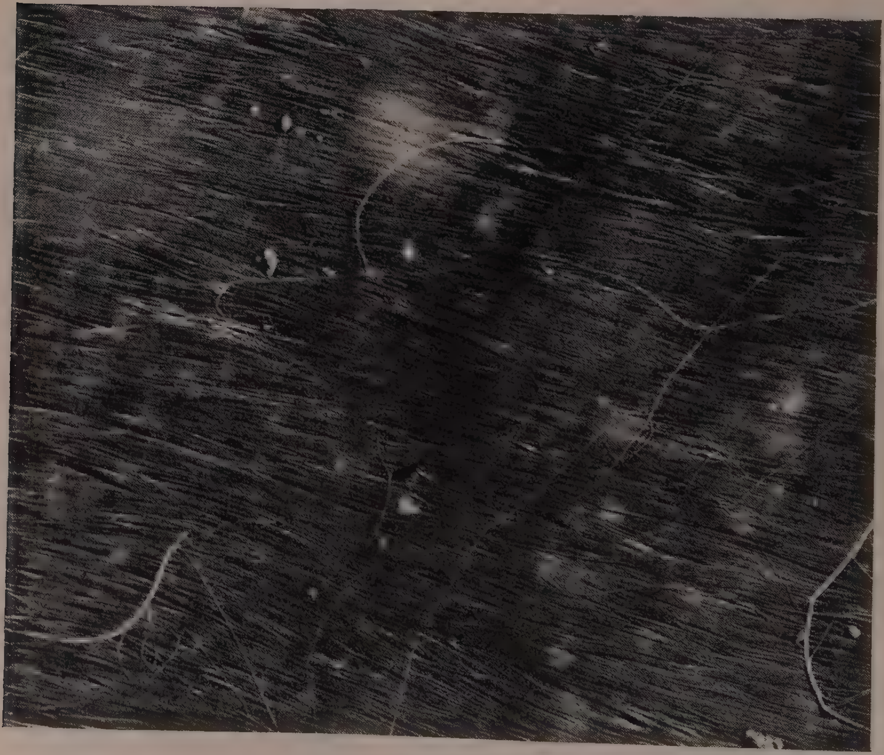


FIG. 2

R. D. PRESTON AND B. KUYPER—PLATE II

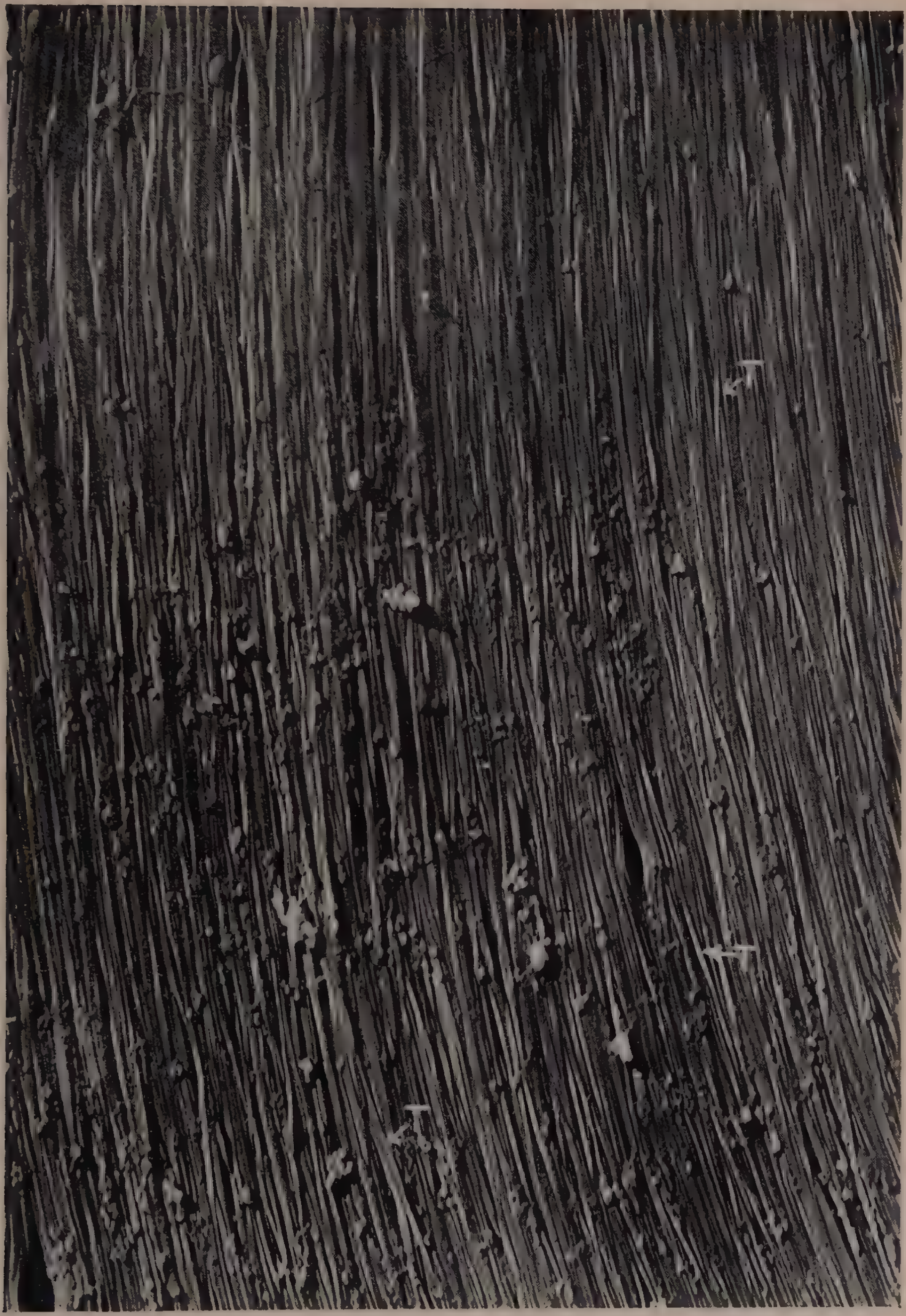


FIG. 1

R. D. PRESTON AND B. KUYPER—PLATE III

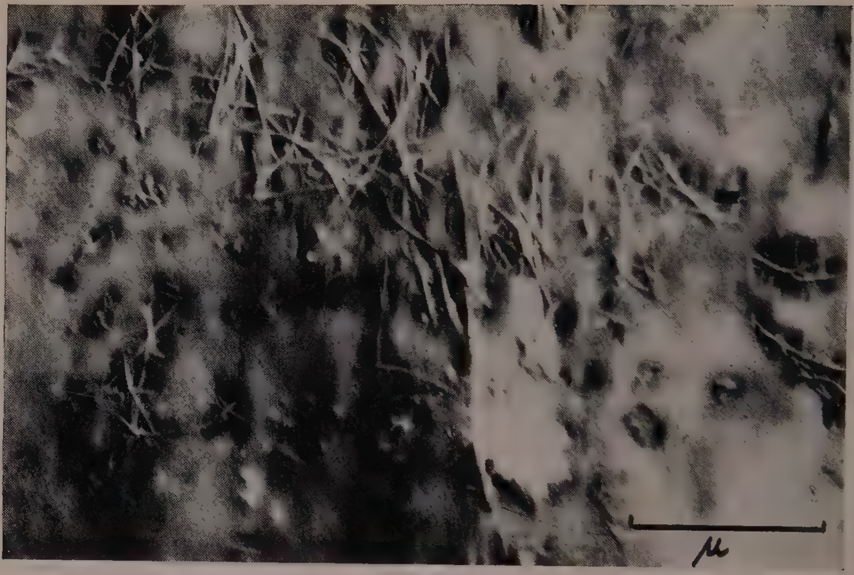


FIG. 1

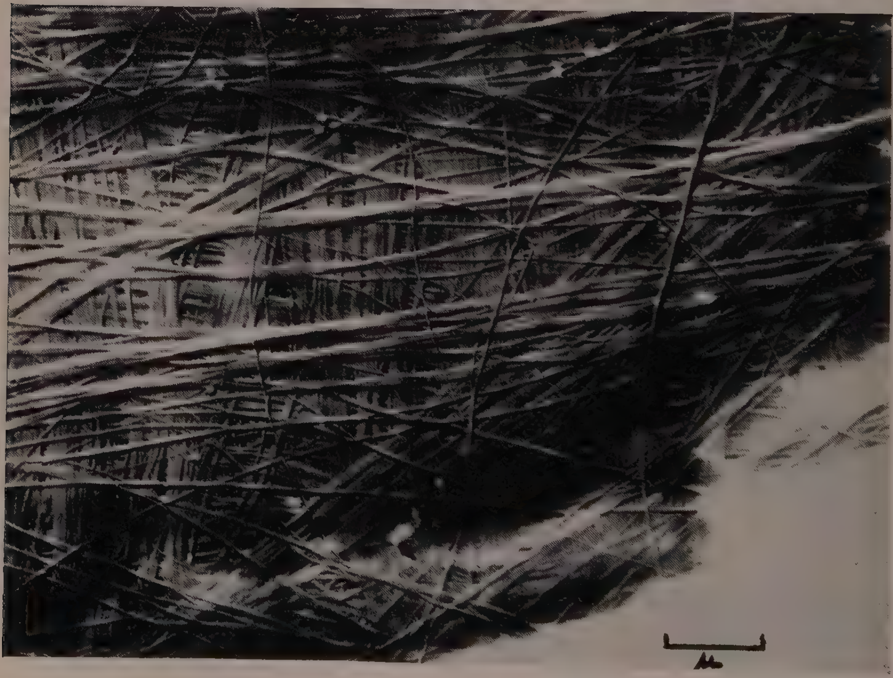
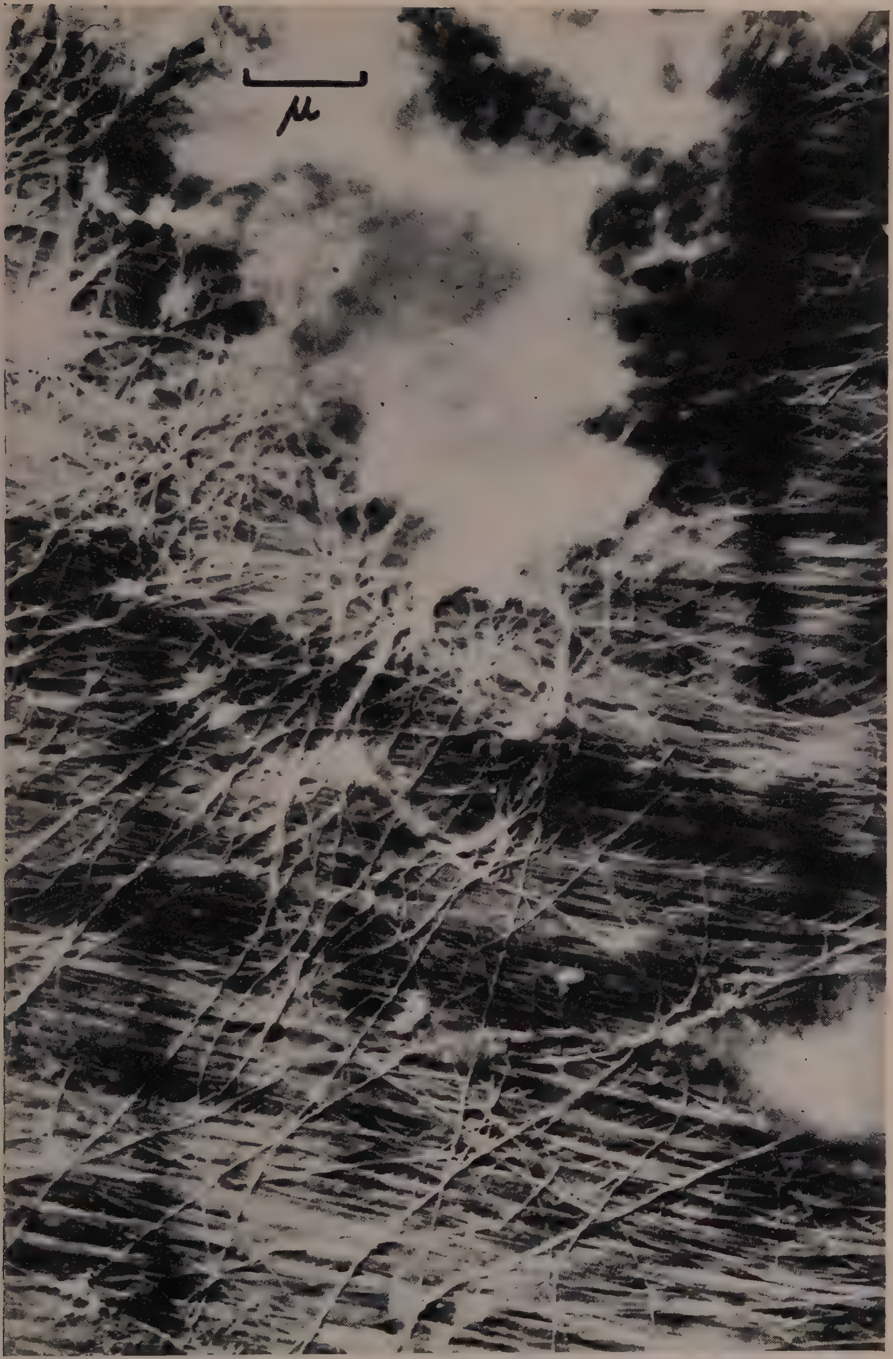
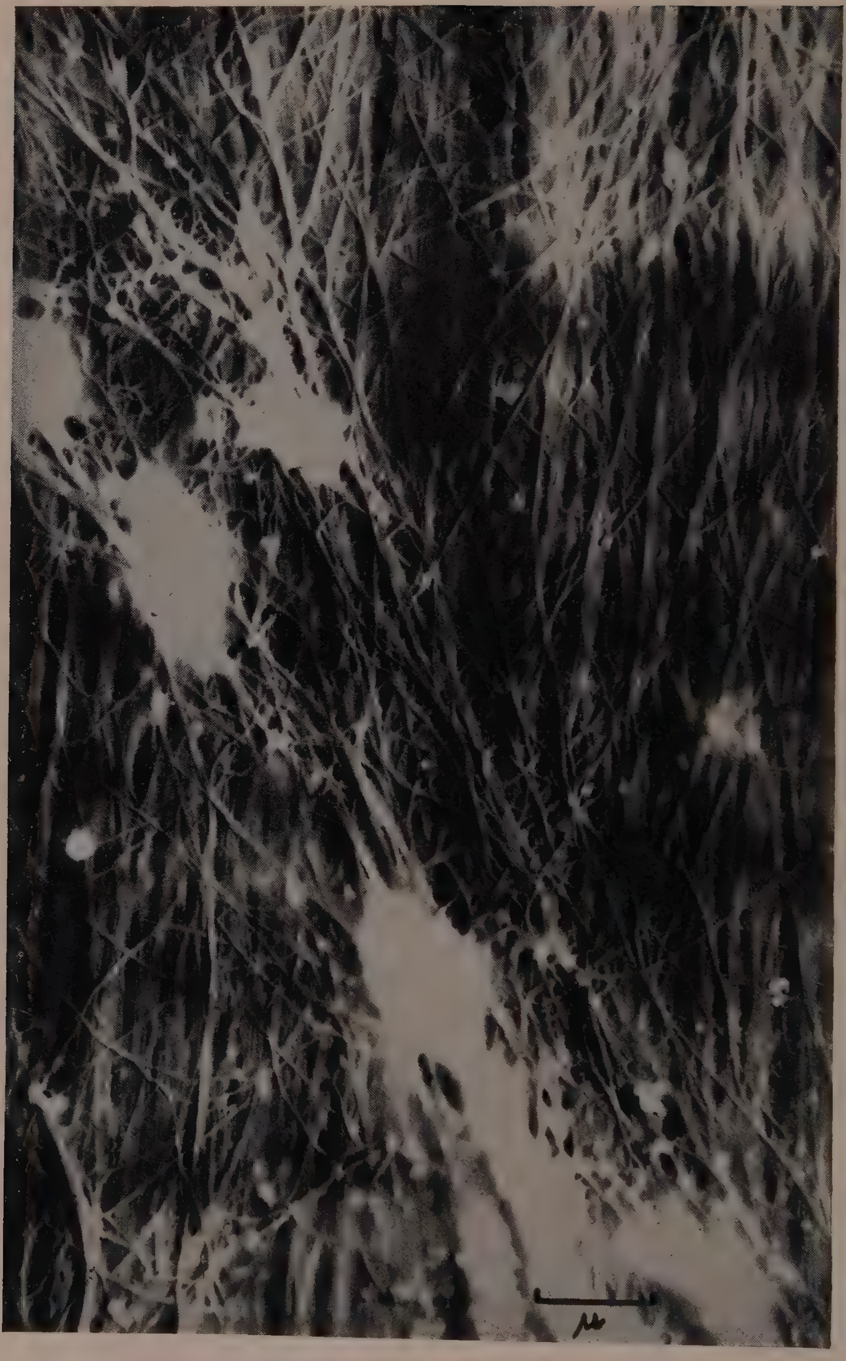


FIG. 2

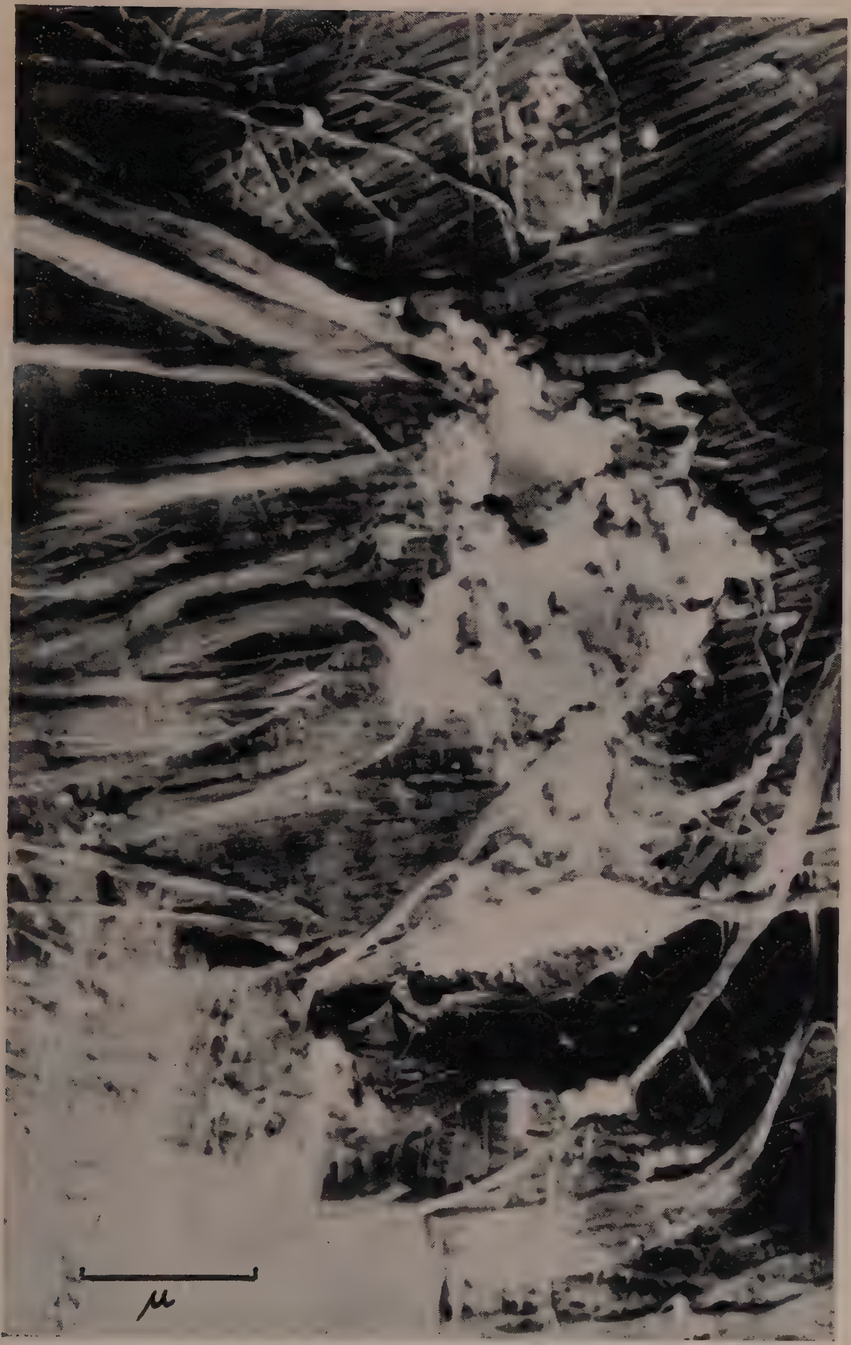
R. D. PRESTON AND B. KUYPER—PLATE IV



R. D. PRESTON AND B. KUYPER—PLATE V



R. D. PRESTON AND B. KUYPER—PLATE VI



R. D. PRESTON AND B. KUYPER—PLATE VII

Analysis of the Exudation Process in Tomato Plants

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SUMMARY

We started from the supposition that the exudation is brought about by two processes: (1) an active transport of salt to the xylem, the 'salt secretion', a process that depends on metabolism and is sensitive to inhibitors, and (2) a water transport effected by the osmotic suction of the sap in the xylem. It was demonstrated that by enhancing the concentration of the medium the exudation rate rapidly decreases. This is followed by a slower rise to a new level which is the consequence of the salt secretion that continues and enhances the osmotic value of the exudation sap. After some time secondary changes of the exudation rate appear. In lowering the concentration of the medium identical changes occur, but now in the reverse direction. By tracing the influence of addition of substances different in mol. weight and lipoid solubility it could be established that the gradual change was the result of salt secretion and not of permeation of the added substance. From this simple supposition about the causes of the exudation process we derived formulae for calculating the salt secretion, the osmotic value of the exudation sap, and the conductivity for water of the root system. Though most observations are in harmony with this concept, there is a difficulty that too low a value is mostly found for the osmotic value of the exudation sap. The possible causes of this deviation are discussed. There is no necessity to accept an active water secretion as well as active salt secretion since salt and water transport cannot be separated.

Important factors in exudation are water conductivity of the protoplasm and salt secretion into the xylem. Both factors are influenced by the osmotic value of the outer solution (D.P.D. of the medium) and by the presence of ions in the outer solution. The two factors must have an identical influence on the transport of water into the transpiration stream.

INTRODUCTION AND LITERATURE

THE literature on the exudation phenomenon is very extensive. A summary of it was given by Kramer (1945 and 1949). We will restrict ourselves to the exudation phenomenon in herbaceous plants. A number of investigators assume that the root works as an osmometer and that water is sucked up as a consequence of the difference in concentration between the sap in the vessels and the outer solution. This will lead to a dilution of this sap and for continuance of exudation it is necessary that osmotic material should be introduced into the vessels. In herbaceous plants it was nearly exclusively salts that were found in the exudation sap (Grossenbacher, 1938, 1939; van Overbeek, 1942; Lundegårdh, 1950). Ewart, Atkins, Priestley, Sabinin, Crafts, and Broyer support the osmotic theory.

Journ. of Experimental Botany, Vol. II, No. 6, pp. 257–297, September 1951.

Sabinin (1925) worked with herbaceous plants. If exudation is an osmotic process the equation $b = k\ (P_x - P_e)$ would hold good, i.e. exudation, b, would be proportional to the osmotic concentration difference between exudation sap, P_x, and medium, P_e. He thought he could prove this formula by determining the rate of exudation on water and on media of various concentration. If A = rate of exudation on water and B = rate in non-permeating solution, he calculates P_x from the formula

$$P_x = \frac{A}{A-B} P_e.$$

The cryoscopically determined osmotic value of the exudation sap being equal to the P_x calculated in this way, he considered this a proof of the osmotic nature of the exudation process. There are, however, objections to this method of calculating the osmotic value of exudation, since the osmotic value of the exudation sap is readily changed when the plant is placed in an osmotic solution.

Laine (1934) found that the volume of the exudation sap is a function of the concentration of the medium $1/V = kC^{1/n}$. He specially examined the cations. The concentration of a cation in the exudation sap acts in accordance with the adsorption formula of Freundlich $s = kC^{1/n}$. In this C is the concentration of the cation in the medium.

It has been known for a long time that oxygen is needed for exudation, and that narcotics and inhibitors for the dissimilation processes also inhibit exudation (Heyl, 1933; Speidel, 1939; Lundegårdh, 1949; Rosene, 1944; van Overbeek, 1942, and others). This points to a relation with vital processes, especially with respiration. Went (1944) pointed to the necessity of sufficient sugar in the root.

As to the cause of exudation van Overbeek has a different view. He put the decapitated tomato plants in water and added mannitol solution until exudation stopped for at least 5 minutes. Then he put plants in water and collected exudation sap. He found that the osmotic pressure of the exudation sap was lower than that of the external solution which just prevented exudation. He concluded that exudation is not caused merely osmotically but that moreover an active exudation force of more than 1 atm. is present. This was sensitive to KCN and considered to be an active water secretion connected with respiration.

Eaton (1943) rejects the notion of an existence of other forces than the osmotic one in exudation. Van Overbeek's experiments, in which exudation is compensated by adding mannitol to the medium solution, he does not consider convincing, because he thinks that mannitol permeates into the root as sugar does. In consequence of this a higher mannitol concentration would be needed than if no permeation occurred.

Hoagland and Broyer (Hoagland, 1944) consider root pressure as a direct result of salt accumulation from the medium by metabolically governed processes of salt movement in the root which were examined quantitatively by them. Crafts and Broyer (1938) assume that the ions in the well-aerated cortex cells are accumulated and transported to the less aerated stele, in which, through the lack of sufficient oxygen, the salts are secreted to the xylem vessels.

Lundegårdh (1943) considers exudation as mainly an osmotic phenomenon. According to him the process of salt secretion is connected with anion respiration which results in polar transport of anions, and he deems it possible that a polar water transport of non-osmotic nature is present.

In 1945 Lundegårdh published a comprehensive investigation on exudation in wheat in which he arrives at entirely new views. The osmotic suction of water by the exudation sap is only of slight importance. The parenchyma cells of the stele exude a salt solution to the xylem. They have a fundamental function in the process of exudation. 'The hydrostatic force of the bleeding P_1 is identical with the turgor pressure of the exuding parenchyma' (1945, p. 109). 'The only reasonable explanation of the transport process is that the anion respiration accumulates the salt ions in the protoplasm and exudes them into the cell sap or transfers them to the next cell' (1945, p. 20). The cations are transferred passively. He also deems it possible that the anion respiration transports water. 'This active transport of water being invariably connected to the anion respiration and anion transport, makes the root to a certain extent independent of the external osmotic suction from the side of the medium' (1945, p. 103).

In 1949 Lundegårdh came to a further modified view. He assumed then that exudation and salt absorption are separate processes. Salt absorption is connected with anion respiration, which is sensitive to KCN and to deficiency of O_2. Exudation is bound to a glycolytic system in which salts are secreted to the wood vessels. It is sensitive to iodoacetic acid and NaF. It is now the cations and no longer the anions which according to Lundegårdh are secreted under influence of processes of metabolism. 'It seems quite feasible to assume that a mechanism situated in the surface of the cell and operating with alternating phosphorylation and dephosphorylation is capable of regulating an exudation of cations from the cell surface.' In every cell accumulation and secretion progress side by side and between them there exists a steady state. In the stele a more active glycolytic system is supposed to be present and consequently the secretion to the vessels is supposed to preponderate. In this revised theory anion respiration continues to play a part in the ion transport.

The reason for the investigation made by us was the wish to submit the osmotic side of the exudation process to a further examination, particularly the question how far it is necessary to assume that water displacement is in addition to osmotic forces also due to secretion. In our investigation it appears that a careful analysis of the exudation process enables us to get a better understanding of various seemingly contrasted results and to sum up the data obtained in a new theory containing elements of the views of most earlier investigators. Though the research has not yet been finished and will be extended in various directions, the results so far attained seemed sufficiently important to us to communicate them here. A preliminary communication on the results obtained in 1949 has already appeared (van Nie, Helder, Arisz, 1950).

EXPERIMENTAL METHODS

The experiments were made with 6-weeks-old tomato plants which had been cultivated in soil and transferred to Hoagland solutions, which were renewed once a week. Aeration of the solutions affected the plants favourably.

The night before the experiment the plants were decapitated and taken to a constant temperature room (24° C.). For the exudation experiment a plant was put in a funnel filled with a solution, the tube of which, directed downward, could be closed with the aid of a rubber tube provided with a pinchcock. During the experiment there was continuous aeration. The surface of the cut was renewed if necessary. By means of a length of rubber tube a curved capillary glass tube with a mm. scale and having a capacity of 11 mm.^{3}/10 mm. was attached to the stump. Every 30 seconds the position of the meniscus was recorded. On changing the liquid in the funnel the old liquid was drawn off through the tube in about 15 seconds. Then we waited about 30 seconds to let the roots drip down. Next fresh liquid was poured into the top of the funnel. The whole treatment lasted at most 1 minute. The exudation fluid was collected in a wide glass tube by means of a rubber tube attached to the stump. In plants bleeding plentifully the exudated fluid amounted to 2 ml. per hour and it was kept in the refrigerator for later examination. Determinations of the freezing-point were made with a microcryoscope from Drucker-Burian and a small model Beckmann thermometer graduated in hundredth degrees.

Two series of experiments were made in 1949, after preliminary experiments had given sufficient orientating data. In 1950 a number of complementary experiments were made. In the first series the progress of the rate of exudation was checked during 8 hours, during which the plant was successively put in various solutions. In this series the plant was transferred to another solution as soon as a new equilibrium had been established in the exudation. Solutions of mannitol, raffinose, urea, thiourea, and glycerol were examined, these substances being added to Hoagland solution in most cases. The plants were kept all day on Hoagland solutions and the difference lay only in the nature or the concentration of the organic substance added. Mannitol was used following van Overbeek and raffinose was also chosen on account of its high molecular weight, which amounts to 594 against 182 for mannitol. This could give us an impression of the effect of permeation of these substances. Urea and thiourea (mol. weight 60 and 76), the latter being more lipoid soluble and also glycerol, mol. weight 92, were likewise examined. In the second series only the instantaneous effect on the exudation, which is produced by taking the plant to a medium of higher concentration, was examined. In the experiments of 1950 the plants were left in the same solution for longer periods in order to trace the further progress of adaptation to a different concentration of the outer solution.

THE COURSE OF EXUDATION ON ALTERATION OF THE OSMOTIC VALUE OF THE MEDIUM

We had got the impression from preliminary experiments that the periodicity of the exudation phenomenon is most pronounced when the plant is in water and this phenomenon prevents one making experiments at various times of the day; consequently in the experiments on the influence of the osmotic pressure of the outer solution we have worked with plants continuously on Hoagland solution. In doing so the plants were constantly provided with salts, so that a gradually increasing deficiency of salts could not be responsible for modifications in rate of exudation.

In the experiment of Fig. 1 the effect was ascertained of adding mannitol to the Hoagland solution, thus increasing the freezing-point depression of the solution from 0·050° to 0·137°. As soon as the mannitol is added the exudation is seen to decrease, compare Fig. 1, *AB*. The lowest level is attained in the first period of 30 seconds, sometimes in the second. Next the rate of exudation (per 30 sec.) increases for about 10 minutes. At *C* a new level is reached, which is lower than the level *A* in Hoagland solution. This level may undergo a further change as an after effect of the change in concentration of the medium and of the internal factors which also play a part in the periodicity of the exudation. In the experiment represented in Fig. 1 these internal changes in the mechanism of exudation have been slight. It is true there is a slight difference in the level of the exudation at the beginning and at the end of the experiments; it is 9·6 mm.^3 per 30 sec. at 10 a.m. and 10·6 mm.^3 at 5 p.m.

The above phenomenon of an initially strong decrease of the exudation and then first rapid, next slow, increase to a new level which is found lower than the level on the Hoagland solution has also been noted by other investigators on increase of osmotic value of the medium (Sabinin, 1925; Eaton, 1943).

The extremely rapid and accurate registration obtained in these experiments, however, makes a further analysis of this phenomenon possible. We distinguish three phases:

(*a*) An immediate rapid decrease in rate (pp. 261–8).

(*b*) An ensuing rise to a new level. This will be discussed on pp. 271 and 275–7.

(*c*) Secondary changes during a longer stay on a certain medium. See pp. 279–85.

THE IMMEDIATE CHANGE OF RATE OF EXUDATION DUE TO CHANGE OF OSMOTIC VALUE OF THE MEDIUM

A similar reaction to the one noted on transition to a medium of higher concentration (see Fig. 1), but in the opposite direction, is found on passing from a more to a less concentrated medium. When, for instance, the exudation has been adapted to the new concentration of mannitol in the medium and the plant is returned again to Hoagland solution, we see the reverse of the process

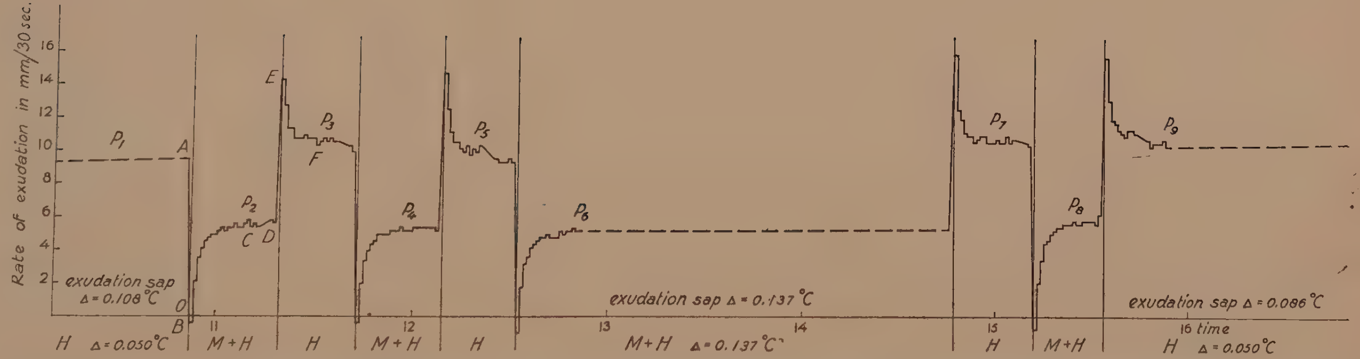

FIG. 1. Influence of changing the osmotic value of the medium on the rate of the exudation. The tomato plant is continuously on a Hoagland solution (H). The osmotic value is increased periodically by adding mannitol (M + H).

described above. First the exudation increases strongly from *D* to *E* in Fig. 1 and rises considerably above the level *A*, which was attained in Hoagland solution at the beginning of the experiment, but after that an initially faster, then slower decrease takes place until the initial level at *F* has been attained again. From the figures obtained in this experiment it appears that the initial decrease *AB* caused by adding mannitol to the medium corresponds with the rapid increase of the exudation *DE*, when the plant is taken back to Hoagland solution.

TABLE I

Difference between sudden decrease and increase of rate of exudation, when changing the osmotic value of the outer solution. Alternately a Hoagland solution and a Hoagland solution of which the osmotic value is enhanced by substances as indicated

A. *Experiments of Type A* (Fig. 1)

Experiment	Substance added	Decrease	Increase	Difference
1	Mannitol	10·0	11·1	−1·1
		11·2	15·4	−4·2
		7·4	12·0	−4·6
2	Mannitol	10·4	9·7	0·7
		10·6	9·8	0·8
		11·0	11·8	−0·8
		11·9	10·4	1·5
3	Urea	6·0	6·0	0·0
		4·8	4·4	0·4
		2·8	4·8	−2·0
4	Mannitol	9·0	8·4	0·6
	Raffinose	5·7	6·7	−1·0
	Thiourea	7·8	6·6	1·2
	Raffinose	4·6	5·6	−1·0
	Mannitol	7·9	7·1	0·8

B. *Experiments of type B* (Fig. 2). Only the differences are indicated for the morning—and the afternoon sets

	Experiment							
	1		2		3		4	
Substance added	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Mannitol I	−0·9	0·4	0·9	−0·7	0·8	1·9	−4·3	−0·9
,, II	1·8	1·4	1·0	0·5	−0·6	0·5	1·9	4·0
,, III	−1·1	0·2	0·5	−1·4	−1·4	−0·1	1·0	−0·7
,, IV	0·8	−0·5	1·1	0·5	0·2	−1·3	0·8	−3·6
,, V	0·0	−1·0	0·9	0·1	—	—	−0·7	0·5

Mean difference: 0·06. Standard deviation: 1·47. Probability: 0·97.

In Table I A and B a number of values for the decrease and increase of exudation on changing the osmotic value of the medium have been brought together. It appears that, considering the errors made in these determinations, the increases and decreases are equal. We shall discuss cases in which they differ on p. 282. The mean difference of the values of Table I B is 0·06, with a standard deviation 1·47. In order to get an insight into the relation between the change in osmotic pressure of the medium and the degree to which the exudation changes, five determinations with mannitol solutions of various concentrations were made one after the other. As the only reason for doing so was to determine the extent of the decrease of exudation, the establishment of a new equilibrium was not waited for (Fig. 2). The plants only remained in the solution containing mannitol till the decrease had reached its maximum and were then returned to Hoagland solution. Next we waited till the level of equilibrium had been recovered and the plant was put again in a solution containing mannitol, but now of a different concentration. In this way five determinations were made both in the morning and in the afternoon. Exudation sap was collected for determining the osmotic value cryoscopically before and after each set of determinations.

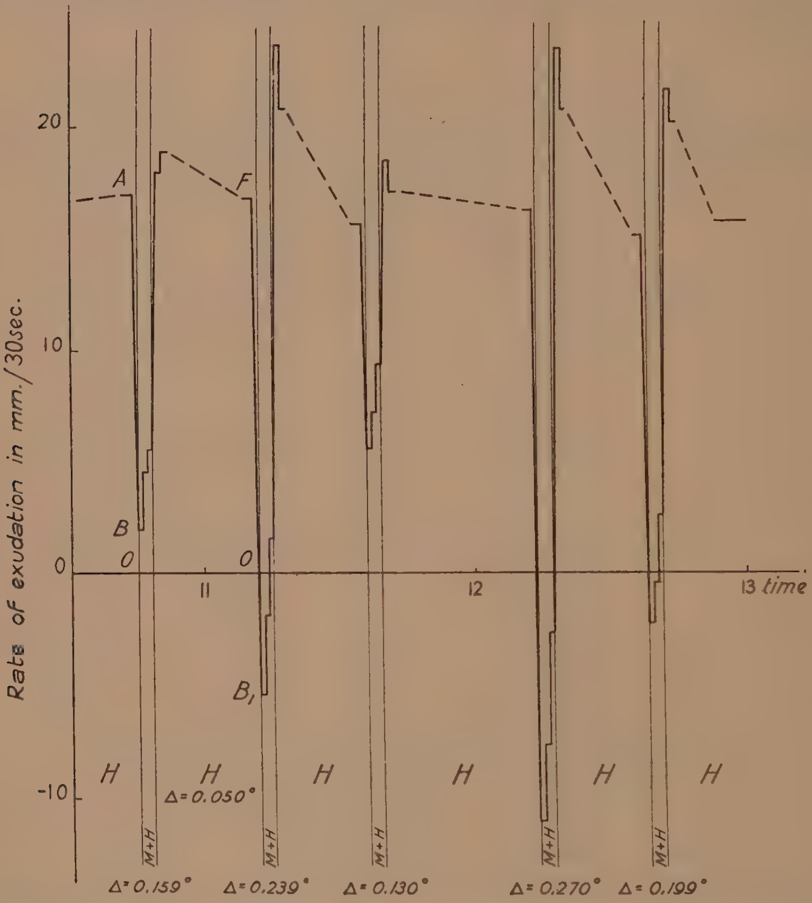

FIG. 2. Sudden changes of exudation rate through addition of different concentrations of mannitol.

Also in these experiments it is desirable that the phenomena of periodicity have an influence as slight as possible. The freezing-point depression of the exudation sap and the mean rate of exudation can enlighten us on this (see Table II). It appears that in various experiments rather important periodic changes occur both in plants in Hoagland solution and in those in water. The most favourable in this respect are the morning-series B_1, B_3, and B_5 and the morning and afternoon series B_4. B_2 shows a faint, but fairly regular periodicity. With these 5 plants therefore 10 series of experiments were made, 5 morning, 5 afternoon series, the results being computed for each series separately.

TABLE II

The osmotic values of the exudation sap, O_b, the compensating osmotic values of the outer solution, comp. O_m, the mean rate of exudation, b, and the salt secretion, S, calculated as $b \times O_b$

	Outer solution		Comp. O_m Δ° C.	Exudation sap Δ° C.	Mean rate of exudation in mm.³/30 sec.	Salt-secretion mm.³ × Δ/30 sec.
		Before set I	—	0·062	20·6	1·28
		set I	0·079			
Exp. B_1	Water	Between sets	—	0·058	17·8	1·03
	Mannitol-sol.	set II	0·053			
		After set II	—	0·040	8·2	0·33
		Before set I	—	0·035	10·3	0·36
	Water	set I	0·065			
Exp. B_2	Mannitol-sol.	Between sets	—	0·050	18·5	0·93
		set II	0·055			
		After set II	—	0·037		
		Before set I	—	0·136	15·9	2·16
	Hoagland sol.	set I	0·180			
Exp. B_3	Mannitol +	Between sets	—	0·138	15·3	2·11
	Hoagland sol.	set II	0·165			
		After set II	—	0·110		
		Before set I	—	0·088		
	Hoagland sol.	set I	0·162			
Exp. B_4	Mannitol sol.	Between sets	—	0·138	16·6	2·29
		set II	0·156			
		After set II	—	0·137		
		Before set I	—	0·040	13·8	0·55
	Water	set I	0·077			
	Mannitol +	Between sets	—	0·037	16·0	0·59
Exp. B_5	Hoagland sol.	set II	0·074			
	Raffinose +					
	Hoagland sol.	After set II	—	0·034	10·7	0·36

In Fig. 3 the results of two experiments have been plotted in a graph. In one experiment the plant had been put on Hoagland solution some time before and mannitol was added to the Hoagland solution, in the second experiment the plant was on water and mannitol was added to the water. Freezing-point depressions of the medium are shown as abscissae, and the corresponding decreases in rate of exudation as ordinates. Through the five points found a straight line can be drawn, giving the regression of the decrease of exudation osmotically effected on the osmotic value of the medium solution. These lines intersect the zero ordinate at $\Delta = 0{\cdot}044°$, $\Delta = 0{\cdot}005°$ respectively. In the four experiments on water the average value was 0·007°, and for those on Hoagland solution with omission of the somewhat deviating afternoon experiment of B_4, 0·050°. For pure water we should have expected the point 0 and for this Hoagland solution the point 0·050°. Taking into account the fairly great experimental errors the correspondence is satisfactory. In particular the rapid change of medium will give no certainty that on transport to a different liquid the solution still clinging to the roots does not slightly alter the concentration of the medium. So it may be concluded that *the decrease of exudation rate is directly proportional to the increase in osmotic value of the medium.*

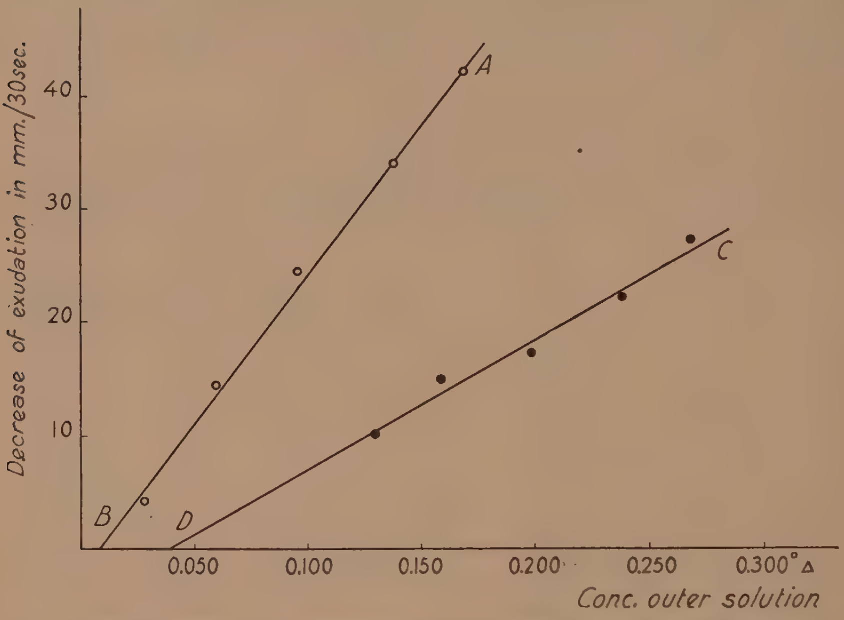

FIG. 3. Relation between the concentration of the outer solution and the decrease of exudation rate. AB, experiment in water; CD, in Hoagland solution. Addition of mannitol in different concentrations.

Since this decrease of exudation is effected in less than 1 minute, it proves that the change of medium immediately affects the exudation. The slope of the regression line for plants in water is steeper than that for plants in Hoagland solution. This indicates that an *osmotic solution of a certain strength has a stronger osmotic effect on a plant in water than on a plant which continues to be on Hoagland solution.* The resistance for water transport is greater in the plant in Hoagland solution than in the plant on water.

From these data it appears that a slight difference in osmotic value of the medium has an immediately notable effect on the exudation. We shall see later on that, likewise for the reverse process, the transport from a more concentrated medium to a less concentrated one, this relation obtains, so that on the ground of the similarity of the effects shown above it may be considered clear that also in the case of decrease of concentration of the medium the increase of exudation is proportional to this.

So it may be expected that small increases in the concentration of the exudation sap also cause a stronger water absorption into this sap from the medium. In analogy with Fig. 3, here, too, it may be expected that for sucking a certain quantity of water from the medium a slighter increase in osmotic value will be needed in the case of the plant in water as compared with the plant in Hoagland solution.

RELATION BETWEEN THE CHANGE OF OSMOTIC VALUE OF THE MEDIUM AND REDUCED EXUDATION

Instead of plotting in the graph the relation between the change of osmotic value of the medium and the resulting exudation decrease or increase, we can also plot the relation between the osmotic value of the medium and the strength of the bleeding left after the decrease. We call this value the instantaneous reduced bleeding, abbreviated b_{red}. When the osmotic value of the medium is greatly increased, bleeding can change into water absorption, which may then be registered as negative exudation. In Fig. 2, on addition of mannitol to a Hoagland solution resulting in an increase in freezing-point depression from 0·050° to 0·159°, we get a positive value *OB* and on addition of mannitol to a $\Delta = 0{\cdot}239°$ a negative value of *OB*. The series of experiments of Table II have been examined with regard to this relation and the regression lines have

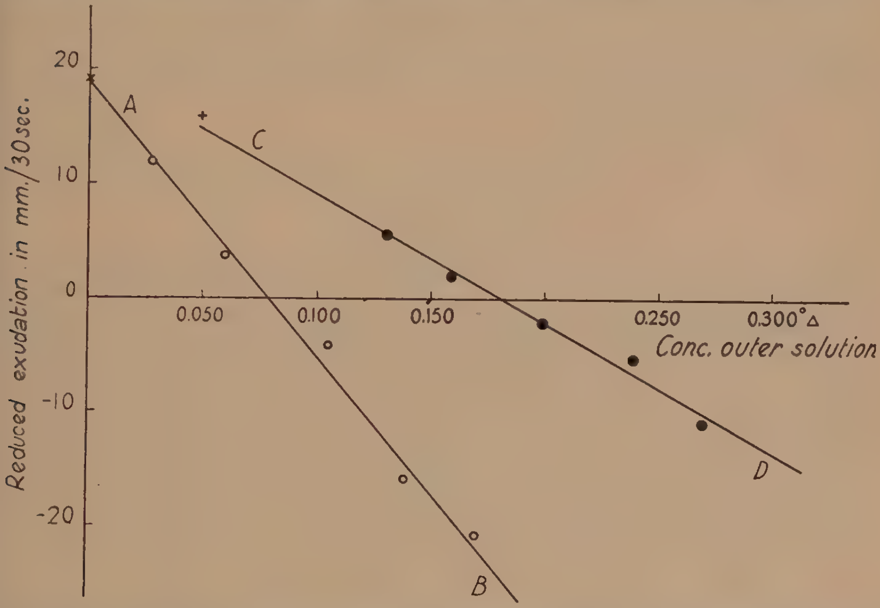

FIG. 4. Relation between the concentration of the outer solution and the reduced bleeding. AB in water; CD in Hoagland solution. Addition of mannitol in different concentrations.

been computed for the reduced bleeding, b_{red}, and the osmotic value of the medium, O_m.

The exudation in the first 30 seconds after the change of the medium solution has been taken as a measure for reduced bleeding. It appears that there is always a rectilinear proportion between b_{red} and O_m.

In formula: $b_{red} = b_0 - aO_m$.

b_0 is a constant having the value of the rate of reduced bleeding when $O_m = 0$, that is, if the plant is in water; a is likewise a constant, and it is a quantity which is a measure for the angle the line forms with the horizontal axis (cf. Fig. 4).

The deviations obtained in some experiments were not great and do not indicate that this linear proportion had better be replaced by a non-rectilinear proportion. Just as we saw in Fig. 3 for the relation between increase of osmotic value of the medium and decrease of exudation, the course of the regression lines in Fig. 4 is different for plants in Hoagland solution and in water. The two graphs are identical and show a rapid osmotic regulation of the process of exudation.

ANALYSIS OF THE SUDDEN AND THE GRADUAL CHANGES IN THE EXUDATION

From the experiments it appears that changes in osmotic value of the medium immediately cause great alterations in the exudation. The relation between the two changes is rectilinear. This indicates a direct osmotic influence of the medium on the xylem sap, in which the root tissue as a whole serves as semi-permeable membrane (Kramer, 1941, 1949). The osmotic value of the exudation sap will also greatly affect the absorption of water from the medium. If we call the osmotic value O_m and O_b respectively, the rate of water-absorption, that is the rate of the exudation b, is related, as far as it is caused by these osmotic forces, to these values as follows:

$$b = k(O_b - O_m) \text{ (Sabinin, 1925).} \qquad (1)$$

In this equation k is a factor which involves properties of the root system, such as size of the water-absorbing part, &c.; the factor k is inversely proportional to the resistance for water-transport and it is therefore a measure for the conductivity for water of the root tissue. As the influence of O_m proved to be great and the same may be expected from O_b, it seems quite possible that the whole water transport in the exudation may be taken as an osmotic phenomenon. Therefore our following derivations will be based on this conception and we shall consider in how far the experimental results tally with this theoretical supposition.

Consequently this implies that by way of working hypothesis we accept Sabinin's formula as the universally availing formula for the whole exudation process.

Seeing that a continuous secretion of exudation sap takes places from the cut xylem vessels in which salt and water are present, it is necessary that absorption of salt from the surrounding tissue into the xylem occurs as well as absorption of water. From the experiments of Lundegårdh, Hoagland, and others it is known that the salt secretion to the xylem is a vital process, i.e. a process connected with metabolism, on account of which the salt concentration in the xylem may be higher than in the outer solution. There is no agreement about the nature of this process. In this publication we will confine ourselves to describing it as an ion transport through the plasm of the root cells without considering where the active force is localized. In most experiments we have placed the plants in a Hoagland solution to make this salt secretion as continuous as possible. Yet during the experiments alterations may have taken place in the salt secretion, which are connected with the periodicity of the exudation or with the changes in osmotic value of the medium.

Under conditions as constant as possible the rate of exudation may be considered constant at least for some time. This is only possible when O_b is constant, which means that a secretion of salt to the xylem takes place at a constant rate.

If we call the rate of salt secretion S, we find that

$$S = bO_b \tag{2}$$

as O_b is proportional to the concentration.

It is obvious that if the salt secretion does not change to the same extent as the exudation as a result of any treatment the osmotic value of the exudation sap would alter, which in its turn would influence the exudation, &c. So a sequence of changes appears until a fresh equilibrium has been attained and the exudation has become constant. For such a period it is possible to give the relation between exudation and time in a formula, if we assume the salt secretion S in this period to be constant.

We call that part of the xylem vessels which is responsible for the osmotic water transport the active volume, V (Fig. 5). The salt secretion S and the water absorption b are probably limited to this region. The quantity of salt secreted in the time, dt, is $S\,dt$. As long as there is a positive exudation, a quantity of exudation sap $b\,dt$ with osmotic value, O_b, is removed at the same time; this contains a quantity of salt, $b\,dt\,O_b$, in which both b and O_b have variable values. The resulting change in the quantity of salt in the volume V is then $S\,dt - b\,dt\,O_b$.

In a state of equilibrium supply and loss of salt are equal. If, however, we increase the osmotic value of the medium, the loss of sap diminishes and the quantity of salt in V increases. On decrease of the osmotic value of the medium the reverse takes place. On dividing the change in quantity of salt in V by this volume, we find the change in osmotic value of the sap in V in the period dt:

$$dO_b = \frac{(S - bO_b)\,dt}{V} = \frac{\{S - k(O_b - O_m)O_b\}\,dt}{V}.$$

After integration[1] we find the relation between O_b and t and after substitution of O_b by b (1), the relation between b and t. The result is for $dO_b/dt > 0$ (this is after increasing the osmotic value of the medium):

$$b = -\tfrac{1}{2}kO_m + \sqrt{\{(\tfrac{1}{2}kO_m)^2 + kS\}}\,\mathrm{tgh}\left(\frac{\sqrt{\{(\tfrac{1}{2}kO_m)^2+kS\}}}{V}t - C\right). \qquad (3a)$$

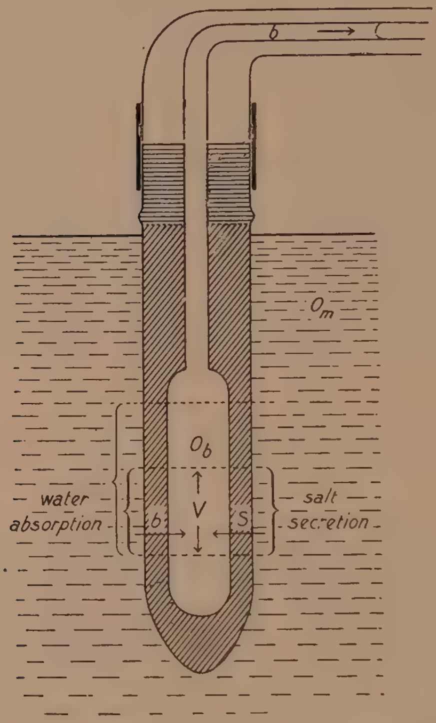

FIG. 5. The root as osmometer. O_m = osmotic value of outer solution; O_b = osmotic value of xylem sap; S = salt secretion; b = bleeding, rate of water absorption; V = active volume of xylem where salt secretion and water transport take place.

For $dO_b/dt < 0$:

$$b = -\tfrac{1}{2}kO_m + \sqrt{\{(\tfrac{1}{2}kO_m)^2 + kS\}}\,\mathrm{ctgh}\left(\frac{\sqrt{\{(\tfrac{1}{2}kO_m)^2+kS\}}}{V}t - C\right). \qquad (3b$$

The two formulae, however, only obtain in case the exudation remains positive. We can, however, increase the osmotic value of the medium in such way that the exudation becomes negative. In that case no loss of salt from occurs. On the contrary sap is supplied at a variable rate but of a constan composition O. So the change in quantity of salt in V is now

$$S\,dt - b\,dt\,O.$$

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[1] We are grateful to Mr. J. H. de Boer for mathematical treatment.

From this follows the change of O_b in V. After integration we again find the relation between O_b and t and with it the relation between b and t. The result is now:

$$b = B - Ce^{-(kO/V)t}. \tag{3c}$$

In this B is the original exudation before the increase in osmotic value of the medium, C is a constant resulting from $b = B - C$ for $t = 0$. So C is equal to the sudden decrease in exudation which occurs on change of medium.

If t increases, b also increases, but not up to the value B as would follow from (3 *c*), as from the moment the exudation becomes positive the course of b is indicated by (3 *a*). On further increase of t the tangent-hyperbola (the same holds good for the cotangent-hyperbola) approaches the value 1. The rate at which this value is attained is inversely proportional to the volume V. So a state of equilibrium is attained in which b reaches a certain value, which follows from

$$b = -\tfrac{1}{2}kO_m + \surd\{(\tfrac{1}{2}kO_m)^2 + kS\}. \tag{4}$$

This formula, which therefore refers to states of equilibrium, may also be derived directly and in a simpler way from (1) and (2). It clearly demonstrates that the exudation is determined by three different factors, viz. the osmotic value of the medium, O_m, the water conductivity of the root system, k, and finally the salt secretion to the xylem vessels, S. Of these the last factor is the driving force of the exudation process. If one or more of these three factors assumes a different value, the equilibrium is disturbed: a period ensues in which b alters as indicated by (3).

In our experiments the osmotic value of the medium was the factor modified. With the aid of the formulae we can now compute the effect of such changes, if k and S are considered constant values, and compare the results with those experimentally found. If the exudation on the solution m_1 was first b_1, it is instantaneously changed into b_2 when the root system is placed on the solution m_2. The change in exudation now follows from (1):

$$b_2 = k(O_b - O_{m2}),$$

$$b_1 = k(O_b - O_{m1});$$

whence

$$b_2 - b_1 = k(O_{m1} - O_{m2}). \tag{5}$$

O_b is constant in this case, because the change is instantaneous. The change is therefore rectilinearly proportional to the change in the osmotic value of the medium, but independent of the absolute value. This has been experimentally found (see p. 266) and was the very reason of our formulating (1). It is, however, important that from these instantaneous changes we can determine the factor k. k is likewise the regression coefficient of the regression lines represented in Fig. 3. It was higher for the distilled water series than for the Hoagland series, which points to a lower resistance in water. It follows directly from the formula that with constant k the sudden decreases must be equal to the sudden increase when a root system is alternately placed in two solutions of different osmotic value. This was indeed found (Tables I A, I B) in the case of a short stay in the solution. In case of a longer stay complications arise owing to the fact that the O_m also affects k and S (see pp. 283, 285).

From the above derivation it also follows that the sudden changes in rate of exudation are independent of the osmotic value of the exudation sap. If there should be another unknown factor, which also affected the process of exudation, we should assume that it does not change or changes instantaneously and linearly with the osmotic value of the medium.

After the sudden change in the exudation a gradual change in the opposite sense takes place, represented by equations 3 *a*, *b*, *c*. In order to compare these formulae with the results we need quantities which have to be derived from the new states of equilibrium. We will therefore restrict ourselves first to those states for which equations 1, 2, and 4 hold good.

In experiment A_2 of Fig. 1 a root system was alternately put on a Hoagland and on a Hoagland+mannitol solution. We know the osmotic values of the solutions O_m. The factor k follows from the instantaneous changes, equation 5. O_b can now be calculated, as it follows from (1) that $O_b = (b/k)+O_m$. From the value of O_b computed, S follows with the aid of (2). The data computed have been summed up in Table III in which the experimentally found and calculated values of O_b and corresponding values of S have been given.

TABLE III

Calculation of conductivity for water, k, osmotic value of exudation sap, O_b, and rate of salt secretion, S, from the data of experiment A_1 (Fig. 1) and comparison between the calculated and determined values of O_b and S. The root system is placed alternately on a Hoagland solution (O_m = 0·050) and a Hoagland+ mannitol solution (O_m = 0·137). The difference in osmotic value of the outer solutions is therefore 0·087

	H P_1	H+M P_2	H P_3	H+M P_4	H P_5	H+M P_6	H P_7	H+M P_8	H P_9
b . . .	9·6	5·6	10·0	5·4	9·4	5·2	10·4	6	10·4
Sudden change .	10·4	9·7	10·6	9·8	11	11·8	11·9	10·4	
k . . .	120	112	122	113	126	136	137	120	
b/k . . .	0·080	0·050	0·082	0·048	0·075	0·038	0·076	0·050	
$O_b = (b/k)+O_m$.	0·130	0·187	0·132	0·185	0·125	0·175	0·126	0·187	
$S = bO_b$.	1·25	1·05	1·32	1·00	1·18	0·91	1·31	1·12	

	Hoagland solution			Hoagland+mannitol		
	Calculated	Determined	Difference	Calculated	Determined	Differenc
Mean value O_b	0·128	0·108–0·086	0·020–0·042	0·184	0·137	0·047
„ S	1·29	0·97	—	1·02	0·71	—
„ b	—	10	—	—	5·7	—
„ k	126	—	—	120	—	—

With these values for k, S, and O_m we want to ascertain in how far equation (3) gives a correct representation of the changing rate of exudation. Fo this purpose we also need the data V and C. We have no experimentally determined V (the active volume) at our disposal. We noticed, however, that th reaching of the constant level of the exudation depends on V. The momen at which this is the case may be determined from Fig. 1. It proves to be on average 10 minutes after changing media (see p. 261). We have now arrived at the following condition:

$$\operatorname{tgh}\left(\frac{\sqrt{\{(\frac{1}{2}kO_m)^2+kS\}}}{V}10-C\right)=1\cdot00.$$

From this it follows:

$$\frac{\sqrt{\{(\frac{1}{2}kO_m)^2+kS\}}}{V}10-C=3. \qquad (a)$$

In order to determine V we have to draw up a second equation. From the data it appears that the exudation after the root system is placed on the mannitol and Hoagland solutions, first for a short time changes into suction. For the following period with increasing exudation, equation (3 *c*) obtains for the first few moments. After an average of 0·7 minute, however, the exudation has already become zero, so that then equation (3 *a*) obtains. Therefore we will restrict ourselves to this. As $b=0$ for $t=0\cdot7$, we find as a second condition:

$$0=-\tfrac{1}{2}kO_m+\sqrt{\{(\tfrac{1}{2}kO_m)^2+kS\}}\operatorname{tgh}\left(\frac{\sqrt{\{(\frac{1}{2}kO_m)^2+kS\}}}{V}0\cdot7-C\right). \qquad (b)$$

From (*a*) and (*b*) an average value of 55·3 mm.^{3} follows for V.

Substitution of this value in (*a*) gives for C: 0·48. The progress of exudation in the period when the root system is placed on the Hoagland+mannitol solution for the second time may, after we have filled up the various values, be given by the simple equation:

$$b=-8\cdot43+13\cdot93\operatorname{tgh}(0\cdot252t+0\cdot48).$$

In Fig. 6 the exudation found in this way has been given as a line and the experimentally found exudation as separate dots.

We see that there is a satisfactory agreement between theory and experiment. Only during the first few minutes the exudation found is a little stronger than that calculated. This, however, might be expected. For in our calculation we started from the salt secretion which takes place on the Hoagland+mannitol solution and which may be derived from the state of equilibrium. Before that the root system was placed on the Hoagland solution in which the salt secretion was much higher. It may therefore be expected that, if we start from the salt secretion in the new state of equilibrium, there will be on the whole a correspondence; only in the beginning of the period the values computed will appear to be somewhat lower than the values found. On our comparing the gradual increases with the gradual decreases we notice that for the decreases the changes are greater in the first few minutes. This is in agreement with equations (3 *a*) and (3 *b*). In case of an increase, exudation follows of a tangent-hyperbola curve; in case of a decrease, part of a cotangent-hyperbola curve.

Summing up, we may say that the course of exudation on transference to a different medium corresponds very well with our view of the process of

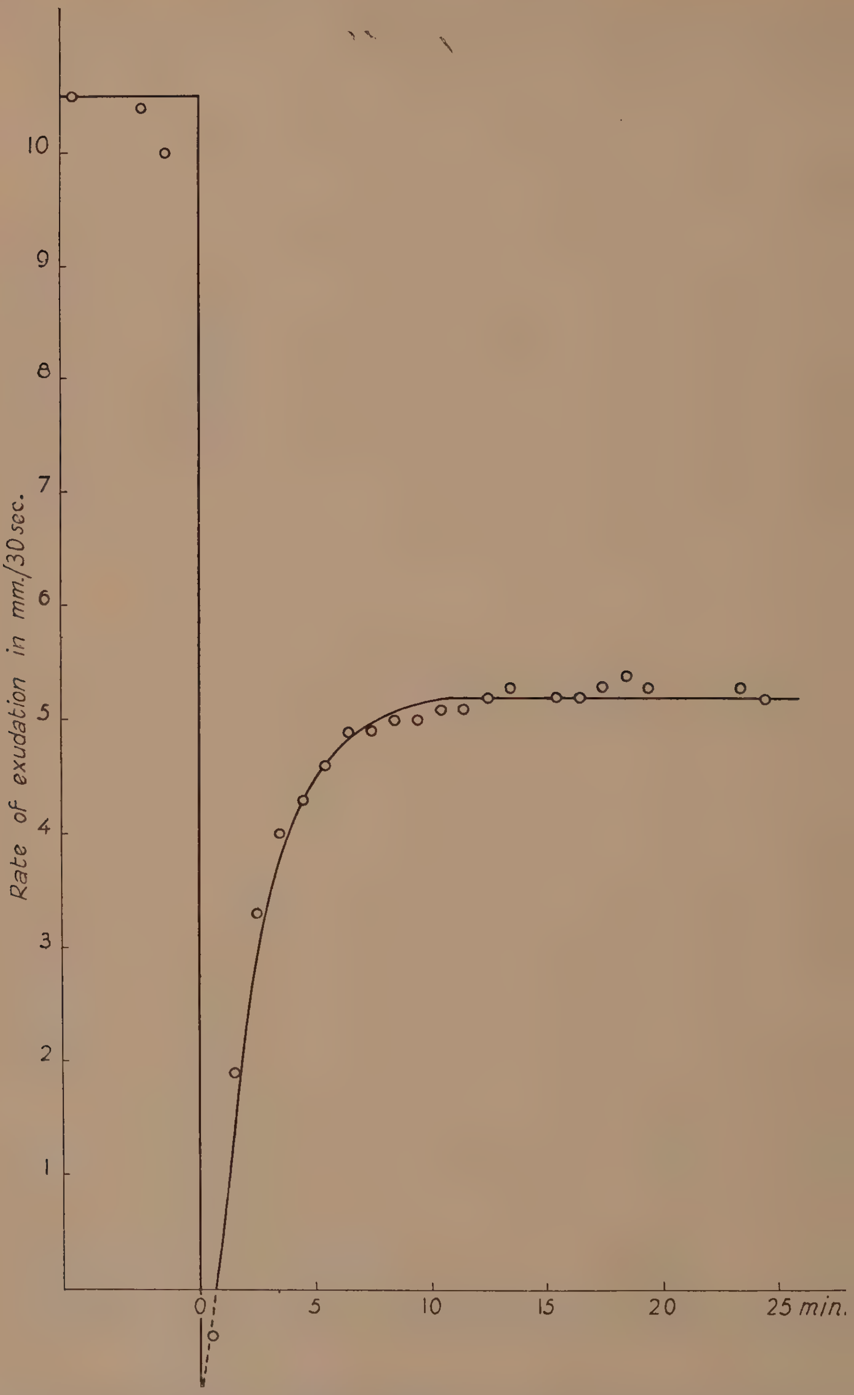

FIG. 6. Comparison between experimental data (o o o o o) and the calculated course of th exudation during the period in which it increases to a new level as a sequel to the salt secretio

exudation and the nature of the instantaneous and the gradual changes in exudation is as expected from the equations.

We notice a difference in the value O_b and S. To this we revert later on. The advantage of this analysis is that it enables us to test in an exact way the behaviour of the various factors playing a part in the exudation, such as the osmotic value of the exudation sap, the water conductivity of the plant, the salt secretion, and the volume of the root active in the exudation. In the subjoined sections we avail ourselves of this method.

OSMOTIC VALUE OF THE EXUDATION SAP

In various experiments exudation sap has been collected and the lowering of the freezing-point of exudation sap and medium determined. In the experiments A_1, A_2, and A_3 of Table IV exudation juice was collected both in the morning and in the afternoon, the plant being in Hoagland solution. In

TABLE IV

Relation between osmotic value of medium and of exudation sap, rate of exudation and salt secretion. Computed values in brackets.

Experiment	Osmotic value of medium Δ° C.	Osmotic value of exudation sap Δ° C.	Rate of exudation mm.^{3}/30 sec.	Salt secretion mm.$^3 \times \Delta$/30 sec.
1949				
A_1	0·05	0·16	15·–	2·4
	0·20	0·19	6·6	1·2
	0·05	0·16	6·8	1·1
A_2	0·05	0·11 (0·13)	9·4	1·0
	0·14	0·14 (0·18)	5·1	0·7
	0·05	0·09	10·5	0·9
A_3	0·05	0·11	7·4	0·8
	0·15	0·14	5·3	0·7
	0·05	0·13	5·7	0·7
B_1	water	0·06	20·6	1·3
	,,	0·06	17·8	1·0
	,,	0·04	8·2	0·3
B_2	water	0·04	10·3	0·4
	,,	0·05	18·5	0·9
	,,	0·04	13·8	—
B_5	water	0·04	16·–	0·5
	,,	0·04	10·7	0·6
	,,	0·03	—	0·4
B_3	0·05	0·14	15·9	2·2
	,,	0·14	15·6	2·1
B_4	0·05	0·09	14·0	2·3
	,,	0·14	17·0	2·3
	,,	0·14	15·0	2·1
1950				
C	0·04	0·18 (0·20)	16·0	3·0
	0·19	0·25 (0·24)	5·0	1·3
	0·04	0·18 (0·17)	13·0	2·4
D	water	0·04	12–14	0·5
	±0·19			
	water			
	0·11	0·12	2·4–0·2	0·15
	water			

between, the plant was put on a mannitol or urea solution and the exudation juice was likewise examined. It appears that in experiment A_1 the osmotic concentration of the exudation sap on Hoagland solution was identical in the morning and in the afternoon, whereas the concentration of the exudation sap after transfer of the plant to a solution of Hoagland+mannitol was higher; the same was found in A_2, while also in A_3, where urea was added, the concentration had increased.

If therefore a plant is put on a solution of higher osmotic concentration, a new equilibrium is reached, in which the rate of exudation has decreased and the concentration of the exudation sap increased.

In the experiments B_1–B_5 the concentration of the exudation sap has been determined at three different times of the day, the plants being either on water or on Hoagland solution. It appears that in these experiments the concentration of the exudation sap on Hoagland solution (B_3 and B_4) is generally considerably higher than in plants on water (B_1, B_2, and B_5), and that fairly great differences in osmotic concentration of the exudation sap occur in the course of the day, which are likely to be connected with periodicity phenomena and are expressed in a proportionally stronger or weaker exudation.

The experiments C and D were made in 1950. In C exudation sap was collected from a plant which was on Hoagland solution; next it was put on a Hoagland+mannitol solution and exudation sap was again collected. Finally the plant was returned to Hoagland solution and exudation sap was collected. The changes in rate of exudation on transfer from Hoagland solution to mannitol+Hoagland solution and back again to Hoagland solution were equally great: k was computed with equation 5. It amounts to 101. We can compute O_b (1) and from this the required salt secretion S (2).

It appears that the computed values of O_b deviate but little from the experimental values. The salt secretion was high in this experiment and became lower when the medium solution got a higher osmotic value owing to addition of mannitol.

In expt. D a plant was put on water, exudation sap was collected, and next the plant was transferred to a mannitol solution ($\Delta = 0{\cdot}190^{\circ}$). As the bleeding was but low, the plant was returned to water and after about 30 minutes to a weaker mannitol solution ($\Delta = 0{\cdot}114^{\circ}$). The exudation was slight and decreased slowly, so that more than 6 hours were needed to collect a sufficient quantity of sap for a determination. We find, just as in B_1, B_2, and B_5, that the salt secretion on water in this experiment is very low, only $\frac{1}{2}$ or $\frac{1}{6}$th of the exudation on Hoagland solution in expt. C. Further, on addition of mannito the salt secretion decreases to a still lower value. k may be computed and is for the change water-mannitol 218 and for mannitol-water 134. As an after effect, k in the transition from water to the second solution with mannitol is still small, 165. Yet all these values are considerably higher than in plants on Hoagland solution.

When in all these cases we compare the Δ of the exudation sap with tha of the medium, it appears that (1) the lowering of freezing-point in plants on water and on Hoagland solution without the addition of other substances is always higher than that of the medium. The difference is greater on Hoagland solution than on water. (2) On addition of mannitol the salt secretion decreases and the exudation falls. To be sure the concentration of the exudation sap on the mannitol solution rises, but not to the same extent as the concentration of the medium has been raised. The lowering of freezing-point becomes nearly equal to that of the medium. The differences are in A_1 −0·009°, in A_2 0·00°, in A_3 −0·009°, in C +0·058°, in D +0·009°. In these the rates of exudation amount to 6·6, 5·1, 5·3, 5, and 2 $mm.^3/30$ sec. respectively. Although in some cases the osmotic concentration of the exudation sap is not higher than the medium solution, yet exudation occurs. (3) The value computed on the basis of the formula for O_b in expt. C corresponds much better with the value experimentally found than the values computed in the previous section for expt. A_2.

The absolute value of the exudation sap collected rose in expt. C to 0·25°, that is about 3 atm. In this case there was still a fair exudation observable. In another experiment we find a still noticeable exudation on a solution with a lowering of freezing-point of 0·28°. So in tomato plants there can certainly be attained fairly high exudation pressures. We now know that to get these we must see that the salt secretion is as strong as possible, whereas the rate of exudation should be kept as low as possible by increasing the concentration of the medium. It is not improbable that under the most favourable circumstances we shall be able to obtain as high pressures as White found in roots.

COMPARISON OF THE OSMOTIC VALUE OF THE EXUDATION SAP WITH THE OSMOTIC VALUE OF THE MEDIUM WHICH COMPENSATES THE EXUDATION INSTANTANEOUSLY

With the aid of the equation $b_{red} = b_0 - aO_m$ developed on p. 268, the value of O_m may be computed for the case where the exudation has been reduced exactly to zero. The determination of the compensating O_m resolves itself into the determination of the point of intersection of the regression line with the zero ordinate in Fig. 4. We call this value the compensating osmotic value of the medium, or abbreviated *comp.* O_m, because at this moment the osmotic suction of the medium compensates the exudation force, so that as a result at that moment no transport of water takes place. One should bear in mind that owing to the continued salt secretion into the vessels the concentration of the exudation sap rises and the exudation continues. If one wants to compensate the exudation at a later moment, higher osmotic concentrations of the medium are needed. Experimentally we are determining the compensation during the first 30 seconds after the change of medium, but if one would compensate a following period of 30 seconds, one would have to take a higher value of the medium.

One might wonder whether this *comp.* O_m coincides with the osmotic value of the exudation sap. In this case the force of the water transport is of an exclusively osmotic nature. Since our object is to compare the *comp.* O_m with the osmotic value of the bleeding sap that exuded before the compensation, it is only permitted to compare the value of the exudation sap with the osmotic value of the medium which instantaneously reduces the exudation to zero. In Fig. 1 the rate of exudation at *A* has a value *AO*, the concentration of the exudation sap being determined by ascertaining the freezing point of the sap previously collected (before *A*). The osmotic value of the medium solution which would make the exudation decrease to zero at *A* is now determined from the regression line. If one waited till the exudation had again risen to *C* and compensated this exudation, then one would have to compare that value with the osmotic value of the exudation sap that had been collected after the equilibrium value had been reached at *C*.

Van Overbeek determined the compensating osmotic value of the medium solution by adding mannitol to the solution in drops. He thought compensation had been reached when the exudation was stopped by the mannitol solution for 5 minutes and he used 15 minutes for the determination. From our Figure 1 it follows that he has not compensated the exudation in *A*, but the exudation at a later moment after an increase of the osmotic value of the exudation sap has taken place. Van Overbeek determined the osmotic value of the exudation sap after the plant had been returned to water. The osmotic value of the bleeding sap then exuding is initially more concentrated and, as appears from Fig. 1, it has not regained the composition that goes with an exudation on water, before *F*. So we can expect the osmotic value of this exudation sap to be lower than that of the compensating osmotic value of the medium, as was found by van Overbeek. He did find great differences indeed. For low salt plants van Overbeek found 0·038° C. for the freezing-point depression of the exudation sap and for the *comp.* O_m 0·156°, for high salt plants 0·109° and 0·227° respectively. These are differences of 0·120° C. or about 1·4 atm.

Also in the experiments of Fig. 2 and Table IV the *comp.* O_m is greater than the value of the exudation sap, which likewise holds good both for plants on water (B_1, B_2, B_5) and on Hoagland solution (B_3, B_4). But the differences are considerably smaller (cf. Table V), for B_1 a.m. 0·019°, for B_5 a.m. 0·038°, for B_3 a.m. 0·042°, and for B_4 p.m. 0·019°. Here we confine ourselves to those observations in which the periodicity has not been too disturbing. These show differences of at most 0·5 atm., but usually much smaller. Suffice it to state this difference here and to revert to the significance and the possible cause of it in the discussion.

It may only be discussed here in how far the difference is based on the fact that the compensation has not been determined at the point *A* of Fig. 2 itself, but during the first 30 seconds following the exchange of medium solution. For, since during a longer period of time after *A* the exudation increases, the O_m will be found to be lower the shorter the period is which is compensated. In order to evaluate the error made here we have determined the regres-

TABLE V

Difference between the osmotic value of the exudation sap and the compensating osmotic value of the medium

	Osmotic value exudation sap			Differences	
	Before compensation experiments	After compensation experiments	Compensating osmotic value		
Experiment	Δ° C.	Δ° C.	Δ° C.	Δ° C.	%
B_1	0·058	0·062	0·079	0·019	24
	0·040	0·058	0·053	0·007	13
B_2	0·035	0·050	0·065	0·023	35
	0·037	0·050	0·055	0·013	24
B_5	0·037	0·040	0·077	0·039	51
	0·034	0·037	0·074	0·039	51
B_3	0·136	0·138	0·180	0·043	24
	0·110	0·138	0·165	0·041	25
B_4	0·088	0·138	0·162	0·049	30
	0·137	0·138	0·156	0·019	12

sion lines for experiment B_3 after various periods of time, viz for the morning experiments after 30, 60, 90, 120, and 150 seconds, and for the afternoon experiments after 30, 60, 90, and 120 seconds. For every regression line the *comp.* O_m was determined. This proved to have a linear relation with the period to which the regression line referred. From this we can compute the *comp.* O_m for a period of time zero. This value, however, differed but little from the value belonging to the regression line of 30 seconds. For the morning experiments the value was 0·179° for a period of 30 seconds and 0·172° for a period zero, for the afternoon experiments 0·163° and 0·162° respectively.

INFLUENCE OF CHANGES IN CONCENTRATION OF THE MEDIUM ON THE FURTHER COURSE OF THE EXUDATION PROCESS

We have seen that the instantaneous increase and decrease of exudation on transfer to and from an outer solution of a different concentration are equal and only depend on the amount of the difference in concentration. This was specially clear in the *B* experiments of Table I B, in which the concentration increase was already followed by an equal concentration decrease after a few minutes. Under these circumstances the factor *k* will be constant. In the *A* experiments of Table I A and in expt. A_2 of Table III there was a longer stay in the higher concentration and the establishment of a new equilibrium was awaited. Here too the differences between increase and decrease of the exudation are slight, though in A_2 there was found a difference in the factor *k*. On Hoagland solution this had an average of 126 and on Hoagland+mannitol solution of 120. These differences become much more striking in experiments in which the concentration of the medium shows a strong increase and the influence of this high concentration lasts a longer time. We will first discuss an experiment in which a plant was successively put for 25 minutes on a

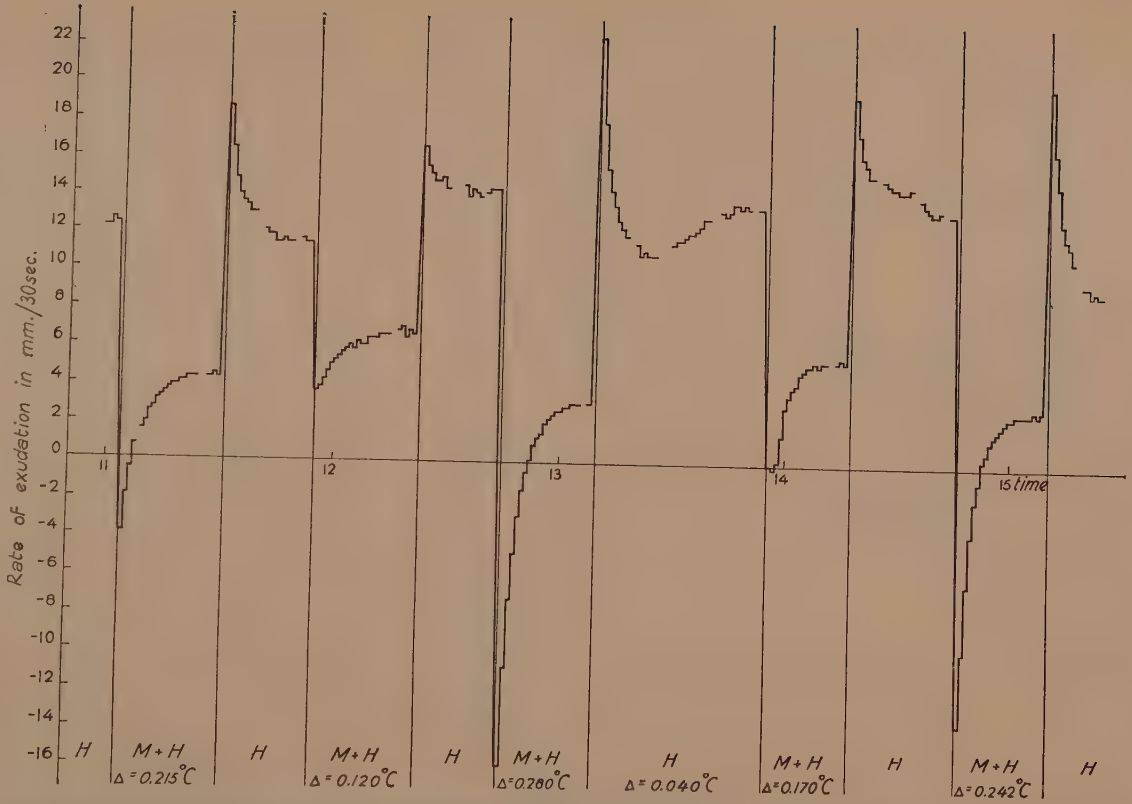

FIG. 7. Influence of successive changes in the osmotic value of the medium on the course of exudation. The osmotic value of a Hoagland solution is increased by adding different concentrations of mannitol.

Hoagland solution of which the osmotic concentration had been increased through the addition of different concentrations of mannitol. The f.p. depression of the Hoagland solution was 0·040° (see Fig. 7). In this way the plant came

TABLE VI

Relation between rate of exudation at equilibrium and osmotic value and √(osmotic value) of the medium

Osmotic value of medium	Rate of exudation	√(Osmotic value)
0·040	126	63
0·120	70	109
0·170	54	130
0·215	44	147
0·242	30	156
0·280	30	167

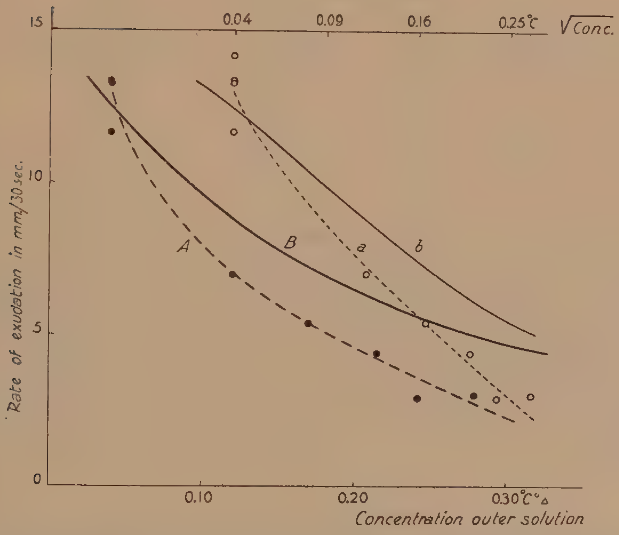

FIG. 8. Relation between osmotic value of the medium and rate of exudation after adaptation to a higher osmotic pressure of the outer solution. Curve *A*, experimentally determined values; *B*, theoretical curve computed using equation (4). The curves *a* and *b* give the relation with $\sqrt{O_m}$.

successively on the following solutions: $\Delta = 0{\cdot}040°$—0·215°—0·040°—0·120°—0·040°—0·280°—0·040°—0·170°—0·040°—0·242° C. After about 15 minutes a new level of exudation had been established. This experiment enables us to determine the relation between the rate of exudation after attaining a state of equilibrium and the concentration of the medium. It is a pity that the data of this experiment are inadequate to compute b with formula (4). In Table VI and Fig. 8 the experimental data have been summed up. In the figure moreover a theoretical curve has been drawn (Fig. 8, *B*) computed with the aid of (4). Here we started from the assumption that the values for k and S on media of higher osmotic values were identical to those on Hoagland solution. In reality these values decrease with an increasing osmotic value of the medium. We may therefore expect that the rates of exudation found (Fig. 8, *A*) are lower than the computed ones.

In the figure the rates computed and found have also been plotted against the root of the osmotic value of the medium (Fig. 8, *a* and *b*). Indeed there is now an almost rectilinear relation between exudation and osmotic value of the medium, as was found by Lundegårdh. This does not mean, however, that Sabinin's formula (our equation 1) is incorrect, as Lundegårdh assumes. For with an increasing O_m the O_b also rises. This fact has been developed in our equation 4.

TABLE VII

Differences between sudden decrease and increase of the rate of exudation of the experiment of Fig. 7

Difference between osmotic values of outer solutions	Δ° C.	Decrease	Increase	Difference
I	0·080	7·8	9·8	2·0
II	0·130	13·6	14·2	0·6
III	0·175	16·4	14·4	−2·0
IV	0·202	27·0	17·2	−9·8
V	0·240	30·2	19·6	−10·6

Table VII gives a survey of the changes in the rate of bleeding caused by a change of the medium concentration. It is clear that the changes of the bleeding, just as in Fig. 3, are proportional to the changes in concentration. But it likewise appears that the decreases, especially in the higher concentrations, are considerably greater than the increases. This must be connected with the longer stay (25 min.) in the solutions. This points to the fact that k, the factor for the conductivity for water, is decreased by a longer stay in higher medium concentrations.

TABLE VIII

Difference between the sudden decrease and increase of the rate of exudation as a result of longer exposure to solutions with higher osmotic values (*same type of experiment as that of Fig. 7 and Table VII*)

Difference between osmotic values of outer solutions	Δ° C.		Decrease	Increase	Difference
I	0·090	a.m.	7·8	8·6	0·8
		p.m.	6·4	6·6	0·2
II	0·111		12·4	11·8	−0·6
III	0·159	a.m.	19·8	13·8	−6·0
		p.m.	19·6	13·4	−6·2
IV	0·217		26·2	16·0	−10·2
V	0·238	a.m.	26·8	18·0	−8·8
		p.m.	27·4	17·6	−9·8

The values of Table VIII refer to a quite analogous experiment to that of Fig. 7 and Table VII in which somewhat different concentrations were used.

Some three concentrations were used both in the morning and in the afternoon. The correspondence between morning and afternoon values is particularly good.

TABLE IX

Changes of factor k, as calculated from the data of Table VIII. In the first column the successive osmotic values of the outer solution

Osmotic values outer solution Δ° C.	Decrease or increase of the rate of exudation mm.^{3}/30 sec.	k
0·040		
0·199	19·8	124
0·040	13·8	86
0·278	26·8	113
0·040	18·0	76
0·130	7·8	87
0·040	8·6	96
0·257	26·2	121
0·040	16·0	74
0·130	6·4	71
0·040	6·6	73
0·151	12·4	112
0·040	11·8	106
0·199	19·6	123
0·040	13·4	84
0·278	27·4	115
0·040	17·6	74

In Table IX the values for k have been computed in accordance with (5) from the changes of the exudation and the concentration changes for the experiment of Table VIII. It appears that on Hoagland solution a higher k value is found. In solutions to which mannitol has been added, k decreases, while in a subsequent stay on Hoagland solution there is even a distinct after-effect, 87, when a very strong solution ($\Delta = 0{\cdot}278^\circ$ C.) has preceded, 120 being the normal value for k on Hoagland solution.

We saw that the changes of the exudation in Table VIII for the morning and afternoon values were equally great and concluded from this that the factor k is unaltered. On attaining the equilibrium level, however, the exudation was considerably lower in the afternoon than in the morning. This shows that in this case the periodicity of the exudation is not based on a change of conductivity for water but on a change in salt secretion.

We will now pass on to the discussion of phenomena which occur when the plants are for a longer time on medium solutions of a higher concentration. Of the great number of graphs which these experiments have yielded, there are several that indicate that, after the state of equilibrium has been reached, the exudation is not constant but slowly decreases in strength. This secondary decrease only appears after 30 minutes or more. In this no doubt the daily periodic course of the exudation acts a part, so that with increasing exudation a fall appears less distinctly, with decreasing exudation on the contrary more distinctly.

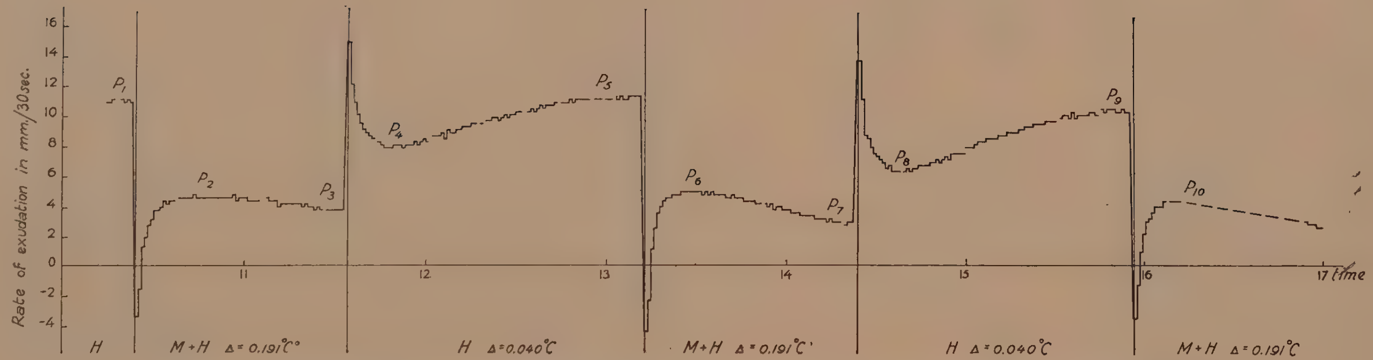

FIG. 9. Rate of exudation of a plant staying for a longer time on Hoagland and Hoagland+mannitol solution.

In Fig. 9 we see a graph of a plant (data in Table X) which was on Hoagland solution and at 10·23 a.m. was transferred to a Hoagland+mannitol solution ($\Delta = 0{\cdot}191°$ C.). After 15 minutes in the state of equilibrium at P_2 the strength of the exudation is 4·6; 50 minutes later at P_3 it has decreased to 3·8. Then at 11·24 the plant is again put on Hoagland solution. The increase in exudation on transfer to Hoagland solution was 11, while the sudden fall at P_1 was 14.

TABLE X

Calculation of k, O_b, and S at different moments P_1, P_2, &c., of the experiment of Fig. 9. The plant is successively put on Hoagland solution ($\Delta = 0{\cdot}040°$ C.) and mannitol+Hoagland sol. ($\Delta = 0{\cdot}191°$ C.). (For calculation of these factors see Table III)

	Hoagland	H+mannitol		Hoagland		H+mannitol		Hoagland		H+ mannitol
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
b . .	11	4·6	3·8	7·8	11·2	4·8	2·8	6·2	10·2	4·4
Sudden change .	14	—	11	—	15·6	—	10·8	—	15·8	—
k . .	92	92	72	72	104	104	72	72	104	104
b/k . .	0·120	0·050	0·053	0·108	0·108	0·046	0·039	0·086	0·098	0·042
O_m . .	0·040	0·191		0·040		0·191		0·040		0·191
O_b . .	0·160	0·241	0·244	0·148	0·148	0·237	0·230	0·126	0·138	0·233
S . .	1·8	1·1	0·9	1·2	1·7	1·1	0·6	0·8	1·4	1·0

This indicates that the conductivity for water has decreased on the mannitol solution, k falls from 92 to 72. After this there follows on the Hoagland solution the rapid fall of the exudation until at P_4 a point has been reached lying lower than P_1. This is connected with the slighter conductivity for water already found at P_3. Now follows a slow rise of the exudation, in consequence of which at P_5 a level is reached of equal height as the one at P_1. If after this the plant is again put on Hoagland+mannitol solution of the same concentration as previously used, the instantaneous fall is 15·6; at P_6 the same state of equilibrium is attained as at P_2. Next, however, the exudation decreases strongly to P_7 which is more than at P_3 in connexion with the decrease in the daily period of exudation. Transferred to Hoagland solution another increase follows and next a fall to P_8. Through a secondary rise P_9 is reached. In this experiment periodicity has had little influence; it has made the level of P_7 a little lower than at P_3; likewise P_8 compared with P_4 and P_9 with P_5. The agreement of the successive observations is striking. In computing the values of k and S after the attainment of the equilibrium we calculated k from the preceding instantaneous change, which can only be approximately correct. The salt secretion is initially strong, then owing to the addition of mannitol it first decreases much, later less. On Hoagland solution the salt secretion is first still low as an after-effect of the previous treatment, but it recovers itself and after a good 90 minutes it has reached its former level; next the same process is repeated on addition of mannitol.

ON THE INFLUENCE OF THE NATURE OF THE SUBSTANCE ADDED TO THE MEDIUM ON THE COURSE OF EXUDATION

Following van Overbeek the preceding experiments have been made with addition of mannitol to the medium solution. As it was conceivable that mannitol solution penetrates into the root (Eaton, 1943; van Overbeek, 1944) and this might affect the progress of exudation, experiments have also been made with other substances. Some investigators (e.g. Sabinin and Eaton) think that the increase of exudation after the sudden strong decrease which we attributed to the temporary salt secretion is the result of the permeating of the added substance into the root. This led to our examining other substances besides mannitol, for it might be expected that the nature of the substance (size of molecule and lipoid solubility) might play an important part in this permeation. Fig. 10 gives the result of an experiment in which we successively investigated the influence of addition of mannitol, raffinose, and thiourea to a Hoagland solution. The osmotic concentrations of these solutions differed but little, so that their effects could be compared. It appears from Fig. 10 that the influence of these substances on the exudation is identical. Since the molecular weight of raffinose is 594, of mannitol 182, of thiourea 76, correspondence is striking. This indicates that the nature of the substance used is of slight importance for the progress of exudation during the first half-hour. It is certainly striking that the exudation on the three substances mentioned is equal in the state of equilibrium, which was certainly not to be expected if these substances permeated in this time into the xylem vessels.

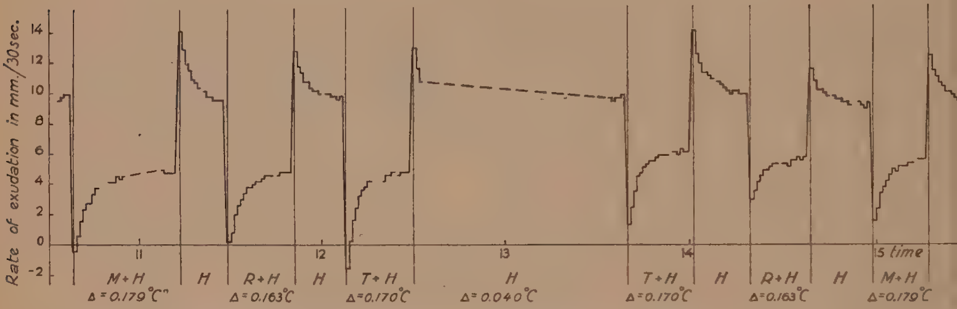

FIG. 10. Comparison of the influence of the addition of mannitol, M, raffinose, R, and thiourea, T, on the course of the exudation.

In order to get some more data the following experiment was made, in which the direct decrease of exudation on addition of five different concentrations of mannitol was compared with that of five different concentrations of raffinose (cf. the type of experiment in Fig. 2). From the regression lines for the relation between concentration and reduced bleeding for the mannitol and for the raffinose solutions the compensating osmotic value of the medium was computed. For mannitol it amounted to 0·071° C. and for raffinose 0·081° C. If this is significant it follows that the *comp.* O_m for mannitol is

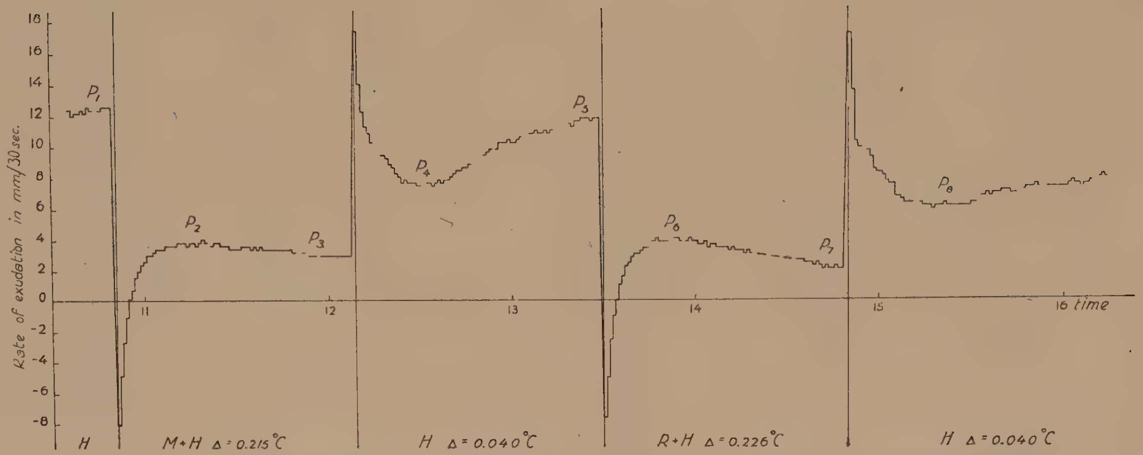

FIG. 11. Long-range experiments with addition of mannitol or raffinose to a Hoagland solution.

somewhat lower than for raffinose. If permeation acted a part here, a deviation in the opposite direction might have been expected. We must therefore conclude that we have found no indication that permeation of mannitol into the root tissue affects the osmotic withdrawal of water from the exudation sap or leads to increase of exudation after the initial rapid fall caused by the higher osmotic medium.

It was interesting to ascertain whether also the secondary changes in the conductivity for water and in the salt secretion which appear owing to a longer stay on media of higher concentration are also found when raffinose is used. In some experiments this secondary influence was hardly perceptible, whereas in others it was similar to that of mannitol. Fig. 11 gives a graph of the exudation in a plant which was successively brought from a Hoagland solution to a mannitol+Hoagland solution ($\Delta = 0{\cdot}215°$ C.), next back to a Hoagland solution, then to a raffinose+Hoagland solution ($\Delta = 0{\cdot}226°$ C.), and again to a Hoagland solution. In this case the secondary change of the exudation has been shown just as clearly for mannitol as for raffinose. For the rest the results of these experiments (Table XI) entirely correspond with those of Fig. 9 and Table X.

TABLE XI

Calculation of k, O_b, and S at different moments (P_1, P_2, &c.) of the experiment of Fig. 11. (Compare Fig. 9 and Table X, for calculation of these factors Table III)

	Hoag-land	Hoagland + mannitol		Hoagland		Hoagland + Raffinose		Hoag-land
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
b	12·6	3·8	3	7·4	11·8	4	2	6
Sudden change	20·6	—	14·4	—	19·4	—	15·2	—
k	118	—	82	—	105	—	81	—
b/k	0·107	0·032	0·037	0·090	0·117	0·038	0·025	0·074
O_m	0·040	0·215		0·040		0·226		0·040
O_b	0·147	0·247	0·252	0·130	0·152	0·264	0·251	0·114
S	1·9	0·9	0·8	1·0	1·8	1·1	0·5	0·7

Experiments made with urea and glycerol, however, indicate that such substances penetrate into the root and can secondarily cause important changes in the exudation.

DISCUSSION

Salt secretion

The investigation concerns the question whether the root can be taken as an osmometer and whether the rate of bleeding is exclusively determined by the difference in osmotic value of the sap and the medium or whether it is determined by quite different processes, such as secretion of water or of a salt solution or both. Meanwhile, however, it has become clear to us that putting the problem like this is not quite correct. For, if the root works as an osmometer, there is also needed, as we have seen, a stream of osmotically active substance, in this case a salt secretion. It is, however, probable that a quantity of water is bound to the secreted ions, so that besides a salt secretion an inherent transport of water takes place. At the same time, however, water will be transported as a result of the difference in osmotic value between sap and medium. And so we see that even if experiments were to show that the rates of exudation are entirely determined by the difference in osmotic value of sap and medium, yet there may be a water transport inherent to the salt secretion. We can therefore agree with Lundegårdh's view, according to which the phenomenon of exudation is based on a secretion process. About the way in which this secretion takes place opinions may vary. Arisz and Heikens (unpublished results) have found that there is a correspondence in structure and function between the salt glands of *Statice*, which secrete NaCl from the leaf, and the endodermis with the cells of the stele in the root which secrete a salt solution into the xylem vessels. Both cell complexes may be considered secretory organs. They are separated from the remaining tissue partly by plasm, partly by an impermeable wall. That suggests that the secretory function of the root is restricted to a complex of cells lying inside the endodermis sheath. The salt from the external solution can penetrate through cell wall and plasm into the endodermis, whence it is transferred to the vessels by secretion. Van Andel, Arisz, and Helder (1950) have pointed out that the root takes up ions from the medium in three different processes. They can be fixed (1) in the absorbing cells of the root in epidermis and cortex, and eventually accumulated in the cell sap (Arisz, 1945), or (2) carried to the zone of growth of the root, where they are used up and bound in anabolic processes, or (3) carried in radial direction to the endodermis and secreted into the xylem vessels. This is the process of salt secretion. In submerged excised root systems the xylem vessels are open, so that we have to deal with processes (1) and (2), for which the strength of (1) will be higher in low salt plants than in high salt plants. In high salt plants, as the tomato plants examined here, the processes (2) and (3) will be predominant. In how far salt transport from the root to stalk and leaves can take place through the sieve tubes must be left out of consideration, the views on this point being conflicting. If such a transport should not take place, the salt secretion into the vessels would be the only way in which the shoot is supplied with salt.

In exudation the secretion of salts to the xylem will be based on the third process. If the medium is water, the salt secretion to the xylem must take place without simultaneous salt absorption from the medium. In this case the salts are withdrawn from the plasm or cell vacuoles. If the medium is a salt solution, the concentration of salt ions in the plasm will be maintained by means of a supply from the medium. Lundegårdh started from the idea that the salt absorption by the root and further transport would be one process of anion transport. Initially he did not distinguish different processes. In his older publications exudation is also regarded as due to this same process, but in his later investigations he considers the salt secretion to the xylem as cation secretion, connected with glycolysis, and not an anion secretion connected with the anion respiration. So on the ground of biochemical differences he has come to distinguish the processes of salt absorption and transport from salt secretion. Arisz (1945) pointed out the likelihood that the surface layer of the protoplasm does not inhibit the passage of ions. Phenomena of exchange and exosmosis of ions, particularly those with radioactive ions, point to this. If anywhere in the symplasm of the root ions are withdrawn or bound by a process, this will lead to an ion displacement in the symplasm and an absorption of ions from the medium. A confirmation of this view was found for leaves of *Vallisneria* (Arisz, 1948), where a leaf length on exposure to light secretes chloride ions into the vacuole and withdraws them from the plasm and the medium. If only one part of a leaf is in touch with the medium and remains in the dark, while another part which is not in direct touch with the medium, but to which the ions have to be supplied via the first part, is exposed to light, this exposed part gets more chloride than the first. This points to a transport through the plasm outside the vacuole in the darkened cells and a salt secretion into the vacuole of the exposed cells, in which the secretion mechanism must lie in the plasm bordering the vacuole. From the sensitiveness of the transport for wounding it appears that the transport must take place in the plasm rather than in cell walls. So there arises a salt transport in the symplasm on account of the secretion of salt into the vacuole by the exposed cells. A similar transport might take place in the root. Besides secretion in the vacuoles of the cortical cells, growth processes in the apex of the root which bind ions, or the salt secretion of the central cylinder which secretes ions to the xylem, may cause ion transport in the symplasm. If we assume this, it is possible that salt ions in the plasm are moved in any direction (cf. Wiersum, 1947, and Helder, unpublished results). New experiments of Broyer (1950) with radioactive bromine likewise indicate that in exudation salt is transported to the xylem through the plasm by-passing the vacuoles of the cortical cells. Broyer, however, does not mention our view. He writes of 'migration along the symplast circumventing, in a measure, movement into or through intervening vacuoles'.

In our investigation it appeared that the strength of the salt secretion into the xylem was dependent on the medium on which the plant is found; on Hoagland solution the salt secretion is much stronger than on water, while as may be understood the salt secretion on water is as good as stopped after some time. Evidently the quantities of salt present in the vacuoles are not freely available for translocation to the xylem. On increase of osmotic concentration of the medium the salt secretion decreases first rapidly, afterwards more slowly. This may be of ecological interest in plants for which the water absorption has been hampered by the condition of the soil (Kramer, 1941). We have not yet investigated how far this is connected with the greater work that has to be done for secretion in a more concentrated solution.

Osmotic regulation

We have found that exudation is instantaneously affected by change of concentration of the medium and when the osmotic value of the medium exceeds a certain value it changes into water uptake by the stump. On the plant passing from a more concentrated to a less concentrated medium an instantaneous increased exudation is likewise found. So it cannot be doubted that the water movement between medium and xylem vessels is osmotically regulated in an accurate and rapid way.

In order to ascertain whether exudation may be considered an osmotic phenomenon, we have started from Sabinin's formula: $b = k(O_b - O_m)$. From this, equation (5) (p. 271) was derived, showing that change in rate of exudation is proportional to change in concentration of the medium. In deriving this we considered O_b not to be changed on transfer to a new medium. The formula, indeed, only obtains for the sudden change in the rate of exudation because as a result of the altered rate O_b is also going to change. This change is initially strong, then slighter. This is clearly demonstrated by the course of exudation. It also follows from the formula indicating the change in the osmotic value of the exudation sap (p. 269).

$$dO_b = \frac{S\,dt - bO_b\,dt}{V}.$$

It is obvious that the rate of the change is connected with the difference in supply and loss of salt and, of course, with the volume in which this occurs. If the loss is lower than the supply of salt, O_b increases and together with it b and consequently the loss of salt $bO_b\,dt$.

The difference between $S\,dt$ and $bO_b\,dt$ decreases rapidly, and together with it the rate at which O_b changes is likewise lowered.

From the above it follows that the formula used by Sabinin for determining the force of exudation (cf. p. 258) is correct only for instantaneous changes.

Strictly speaking we did not examine the instantaneous effect in our own experiments, since the changing of solution always requires some time. By working quickly, however, this error may be kept slight. So we found in determining the compensating osmotic value of the medium that only a slight correction was required. Therefore we used formula (5) for computing k, he conductivity of the root for water transport from medium to xylem.

In order to find out whether besides osmotic regulation there are other ransport mechanisms to the xylem, we have followed van Overbeek's method. The osmotic value of the exudation sap was determined cryoscopically and ikewise the compensating osmotic value of the medium solution which brings a stoppage of exudation within 30 seconds. This value was computed from the equation of the regression line (Fig. 4). The values obtained are given in Table V. Considerably smaller differences than those found by van Overbeek were found here, at most 0·5 atm. We have discussed this point more fully on p. 278, and explained it by the better method used for effecting the compensation. There remains, however, a not inconsiderable difference especially in proportion to the osmotic value of the exudation sap which is great enough o justify the question whether this points to a process of active water ecretion.

In Table IV a number of determinations of the lowering of the freezing-point of the exudation sap have been gathered which are compared with those of the external medium. Increase of concentration of the medium is associated with a rise in concentration of the exudation sap, but never to the same extent. The consequence is that in high medium concentrations the two values approach each other, especially in the case of the A series of 1949. The differences found in A_1 and A_3, showing that the lowering of freezing-point o the medium is somewhat higher than that of the exudation sap, are within the limit of error of the determinations. It is striking that nowhere is this limit considerably exceeded. If a strong active water secretion takes place, this might certainly be expected. In our opinion this experience tells against the presence of a strong water secretion in the exudation process. The good conductivity for water of the root would be an exceedingly great impediment fo maintaining conditions at variance with the osmotic equilibrium. It should not be forgotten, however, that it has been stated here that with equal concentration of exudation sap and medium solution exudation can take place

Based on the osmotic theory and with the aid of the formulae derived o p. 271 we have also computed the osmotic value of the exudation sap in some cases (Table IV). For expt. A_2 the values computed were higher than the values found, for expt. C, however, the agreement was better. Summarizing we find that the osmotic theory does give a satisfactory insight into the exudation process, but that there are three items which require explanation:

(1) The osmotic value of the exudation sap computed according to the osmotic theory appears not always to correspond with the values found it is too low.

(2) Also with equal concentration of exudation sap and external solution exudation does take place.

(3) The osmotic value of the external medium which instantaneously compensates the exudation is higher than the osmotic value of the exudation sap.

In each of these three cases therefore the osmotic value of the exudation sap is found too low.

Putting aside the experimental errors, which are certainly not inconsiderable, two explanations are possible.

In the first place we may doubt if the composition of the exudation sa that appears at the stump is the same as that of the sap present in the 'active volume' of the vessels as it is possible that during the transport upwards sal and water exchanges between sap and adjoining cells may occur.

Owing to salt absorption from the exudation sap, the concentration in those parts of the xylem in which an exchange of water with the external medium i no longer possible might even fall below that of the medium.

In deriving formula (3) we assumed that the salt-secreting zone coincide with the water-absorbing one. If, however, the latter is larger and if $O_b > O$ more water will be absorbed, in consequence of which the exudation sa from V will be diluted (Fig. 6). Of course the two processes may co-operate and lead to a 'dilution effect'.

If a dilution effect is out of the question, an extra water-secreting process would have to carry water to the xylem vessels. In our opinion this need not necessarily mean the acceptance of an 'active water secretion', as van Overbeek assumes. For as far as we know a secretion of pure water could hitherto not be demonstrated in exudation; always a more or less high concentration of salts occurs. Therefore we deem it possible that salt secretion is also responsible for this process because on transport of the ions from the cortical cells to the xylem vessels water is carried along. This is also Lundegårdh's view. So it is imaginable that with equal osmotic value of exudation sap and external solution exudation does take place. A vital condition, however, is that either this process must be independent of the change in concentration of the medium or it must change rectilinear proportionally with it. Finally, what was already observed at the beginning of the discussion may be repeated here: even if too low values for O_b should not have been found, a water transport inherent to the salt secretion is possible.

Rate of exudation

If the osmotic theory holds good, this is determined by formula (1). As we have seen that this theory cannot fully explain the phenomena, deviations will be found. Lundegårdh found a rectilinear relation between √external O.P. and exudation. His opinion is that this indicates that the theorem of Torricelli for the flux through tubes applies rather than Poiseuille's law. Lundegårdh's observations refer to exudations after a constant level has been attained. For this our formula (4) gives the relation between exudation and osmotic concentration of the external solution. In Fig. 8 the course of the theoretical curve has been given. We saw on p. 282 that, considering k and s become smaller on increasing osmotic value of the medium, a reasonable correspondence obtains between the values found and those computed. Within definite limits there is a proportion between the rates of exudation and $\sqrt{O_m}$. So there is no reason to attach a theoretical significance to Lundegårdh's statement.

Conductivity for water

All investigators without an exception have found that increase or decrease of the osmotic value of the medium is immediately expressed in the rate of exudation. The comparatively great change in rate of exudation through a slight change in osmotic concentration of the medium shows a rather great permeability of the root for water (Eaton, 1943; van Overbeek, 1942; Lundegårdh, 1945). The water can be displaced in either direction in the root. In correspondence with this we found in the experimental part that the rate of exudation reacts instantaneously to an increase or decrease of the osmotic concentration of the medium. The change in rate of exudation is then proportional to the change in concentration of the medium (Fig. 3). The slopes of the lines AB and CD in this figure, however, are different and indicate that with plants in a Hoagland solution a greater change in concentration of the medium is needed to produce a similar decrease in the rate of exudation. This must be due to a lesser resistance for water transport in a plant which has previously been in water than for a plant which has been on Hoagland solution.

A measure for this conductivity of water is the factor k of formula (1). The size of k depends on the volume of the root system, the permeability of the walls, and the permeability of the plasm. Increase of the osmotic concentration of the medium solution has a noticeable influence on the factor k after a short time. This effect is reversible.

It is not clear by what route water moves from the medium to the xylem. Theoretically cell-wall as well as plasm and vacuole of the root cells may be considered for this. It is known that in the endodermis at any rate the plasm has to be passed, but it is unknown whether the plasm of the root cells outside it acts an important part. The influence of salts on the rate of water transport shows at any rate that somewhere these salts affect the water permeability of the plasm. The influence of salts on the permeability of the protoplasm for water was investigated by de Haan in this laboratory in 1931–3 for epidermis cells of onion scales. He found that they affected the water permeability according to a Hofmeister series which was measured from the rate of plasmolysis. Though de Haan examined the system medium-surface layer-plasm-tonoplast-vacuole, whereas in our experiments the system medium-surface layer-plasm-surface layer-xylem vessels has to be considered (transmeability versus tissue permeability, Arisz, 1945), it makes in this case no great difference as de Haan ascribed the permeability changes to the whole protoplasm and not to special layers. This statement was corroborated by Höfler (1950). Other investigators have also found an influence of ions on the water transport in exudation and transpiration (Speidel, 1939; Tagawa, 1934).

Permeation of substances in the root

As stated on p. 261, Sabinin and Eaton likewise found that on putting a tomato plant in a medium of higher concentration the exudation suddenly decreases and increases gradually. We have attributed this phenomenon to the salt secretion which continues and must cause an increase of concentration in the exudation sap on decrease of the water transport. Sabinin and Eaton explain this by permeation of the substance added into the root. To enable us to form an opinion on this subject a number of experiments have been made by us by adding various substances to the medium. These have been described above. Seeing that the addition of quite different substances, as mannitol, raffinose, and thiourea, did not cause any essential change in the progress of exudation, we cannot share Eaton's view. The nature of the organic substances added does not affect the establishment of the new equilibrium. This has been ascertained most accurately for raffinose and mannitol with molecular weights of 594 and 182. Here an indication should have been found that raffinose had a less strong effect or an effect produced more slowly than mannitol. Of course this does not mean that a substance as sugar or mannitol cannot permeate into the root or be taken up into it. Sugar and glucose are certainly taken up into the root. From our experiments it only appears that, if they should be taken up, this would not influence the direct osmotic regulation of exudation. Secondarily the sugars can strengthen the exudation, as has been convincingly demonstrated by Went. Only for glycerol and in a less strong degree for urea we found a deviating course of exudation which indicates that salt secretion and conductivity for water are strongly inhibited here.

Periodicity

The regression lines for the influence of the osmotic value of the medium on exudation (cf. Fig. 4 and Table VIII) show fairly equal slopes in the morning and afternoon series. This means, as we already mentioned, that the conductivity for water in these periods is virtually unchanged. Seeing that exudation in the morning and in the afternoon can show considerable differences, which may be expected in connexion with the periodicity of this phenomenon (Grossenbacher, 1938, 1939), this periodicity will probably be in a less degree related to the changes in the water permeability of the plasm. As exudation moreover depends on the differences in osmotic pressure between exudation sap and medium and therefore on the salt secretion to the xylem, a change in this later factor will probably cause the periodicity of exudation. This subject will likewise have to be further investigated.

The role of factors, influencing exudation, in the transpiration-stream of the normal plant

Most authors do not ascribe an important role to the root pressure in the water transport of the plant. White, who found high pressures in his artificially cultivated tomato-roots, surmises a larger influence. On the basis of the results of this research it is not likely that in a normal plant high root pressures occur. Though we have found rather high osmotic pressures, these are produced under circumstances unfavourable for water transport. We saw that the osmotic value of the xylem sap is a function of the salt secretion and the rate of the water transport. It is highest when salt transport is at a maximum and water transport at a minimum. A continuous salt secretion into the xylem will of course result in a continuous water flow, but according to Kramer this is small compared with the transpiration stream.

We have developed a method for determining the conductivity of water and the salt secretion in the root. These factors must influence in the same way the water transport of the transpiration stream in the root. We found that they depend on the ions present in the medium and on the osmotic value of the outer solution. In conclusion we have to point to the remarkable fact that by enhancing the osmotic value of the medium corresponding changes in the rate of the transpiration stream have been found (*Überführungsreaktion* of Renner, 1929; Brieger, 1928; Peris, 1936). Though these potometer experiments are more complicated they point to the same phenomena. We limit ourselves here to the mention of the similarity of these processes on which further research is needed.

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The Isolation of *l*-Quinic Acid from the Apple Fruit

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SUMMARY

1. A method is described by means of which several grammes of *l*-quinic acid were isolated from young fruits of the Worcester Pearmain apple stored for a number of days in nitrogen.

2. The essential stages in the method are the removal of colouring matter from extracts of the frozen ground pulp tissue of the fruit with charcoal, the removal of amino acids by absorption on cation exchange resin, and the separation of the organic acid from sugars by adsorption of the former on anion exchange resin. Finally, the quinic acid was separated from malic acid by fractional displacement from the anion exchange resin.

3. The characterization of the isolated quinic acid, and of malic acid also isolated from the fruits, is described.

4. Citric acid is shown to be formed by oxidation of quinic acid with hot hydrogen peroxide.

5. The possible function of quinic acid in the apple fruit and in plants generally is discussed.

INTRODUCTION

It has generally been assumed that the only organic acid present in quantity in English apples such as Bramley's Seedling and Worcester Pearmain is malic acid. Tavernier and Jacquin (1947) showed that in six varieties of apples grown in France, 90–95 per cent. of the acid present was malic with small amounts of citric, lactic, and succinic acids. During growth between June and September a decrease in acidity was accounted for entirely as malic acid. The same authors (1948) showed that in seventeen varieties of pears malic and citric acids made up the greater part of the titratable acidity and, in certain varieties, the amount of citric acid was surprisingly high, sometimes exceeding that of malic acid.

In a recent review of the metabolism of organic acids in plants Thimann and Bonner (1950) show the widespread distribution of citric and malic acids in leaves and fruits. Of the other plant acids, oxalic acid is fairly widespread in leaves and tartaric is the main constituent of the fruits of a few genera, notably those of the Vitaceae. Isocitric acid, apart from the peculiar position it occupies in the Crassulaceae, appears only to be present in quantity in fruits of certain of the Rosaceae. Most of the other open-chain acids are confined to and appear to be characteristic of a limited number of genera. Aromatic acids such as 'tannic', gallic, chlorogenic, protocatechuic have been found from time to time in various plants, but their metabolic role has been little studied. The

Journ. of Experimental Botany, Vol. II, No. 6, pp. 298-315, September 1951

same may be said of acids of the cyclohexane series. Thimann and Bonner (loc. cit.), summing up the position, state that 'detailed studies on the biochemistry of organic acid formation and metabolism in fruits are few'.

On examination of extracts from immature Bramley's Seedling apples by means of filter-paper chromatograms, run in *n*-butanol-formic acid-water (40 : 10 : 50 v/v), it was noticed that several spots having an acid reaction to bromophenol blue appeared. In addition to a large spot which appeared to correspond in position to malic acid ($R_F = 0{\cdot}49$) there was another well-defined spot ($R_F = 0{\cdot}18$) and traces of a third spot having an R_F of 0·07 (Hulme and Swain, 1951). The present author had found similar spots in chromatograms prepared from extracts of Worcester Pearmain and Cox's Orange Pippin apples which had been freed from amino acids. In extracts of immature Worcester Pearmains the spot of R_F 0·18 was rather larger than the presumed malic acid spot, more especially if the apples had been stored for a few weeks in an atmosphere of nitrogen before analysis, but no conclusive quantitative data are available on this point.

A search of the literature on the chromatography of the organic acids failed to reveal any acid in the position occupied by this spot in the solvent used. A small amount of acid corresponding to the spot was isolated and a preliminary examination suggested that it might well be a dihydroxy tricarballylic acid (Hulme and Swain, loc. cit.).

Meanwhile the present author was preparing to isolate large quantities of the unknown acid from several kilograms of the pulp tissue of Worcester Pearmain apples (av. wt. 15 g.). These apples had been stored in nitrogen at 15° C. for 3 weeks, but it should be noted that the acid is not peculiar to apples stored in nitrogen, the only reason for using such tissue being the observation mentioned above.

To obtain the acid fraction from the fruit as free as possible from other compounds, colouring matter and amino acids were removed and the acids separated from the large amount of sugars present by absorption on a column of Deacidite E. After displacement with 0·1 N HCl the acid solution was concentrated *in vacuo*. Chromatograms of this concentrated extract, run in butanol-formic acid-water, showed, after spraying with bromophenol blue, a large spot corresponding with a 'marker' spot of malic acid, a spot of the unknown acid approximately a quarter the size of the malic spot, another unidentified spot having an R_F value of 0·07, and traces of spots which were provisionally assigned to citric and fumaric acids.

There was clearly sufficient of both malic acid and of the unknown acid (R_F 0·18–0·22) in this extract for isolation in considerable quantity and the present work is confined to these. A preliminary attempt at separation by absorption of the acids on Deacidite E and collection of the 0·1 N HCl displacement liquid in a number of small fractions showed a considerable overlap of the two acids in the middle fractions, the unknown acid leaving the column first and the malic acid last. The possibility was examined of achieving a better separation by partition chromatography on silica gel columns following the method of Isherwood (1946) and Bradfield (1950). Small-scale experiments (~ 50 mg. total acid, calculated as malic) using methyl *iso*butyl ketone as flowing solvent gave a good separation of malic acid and the unknown acid as well as of the traces of the presumed citric and fumaric acids (see Fig. 1). The acids left the column in the order expected from paper chromatograms,

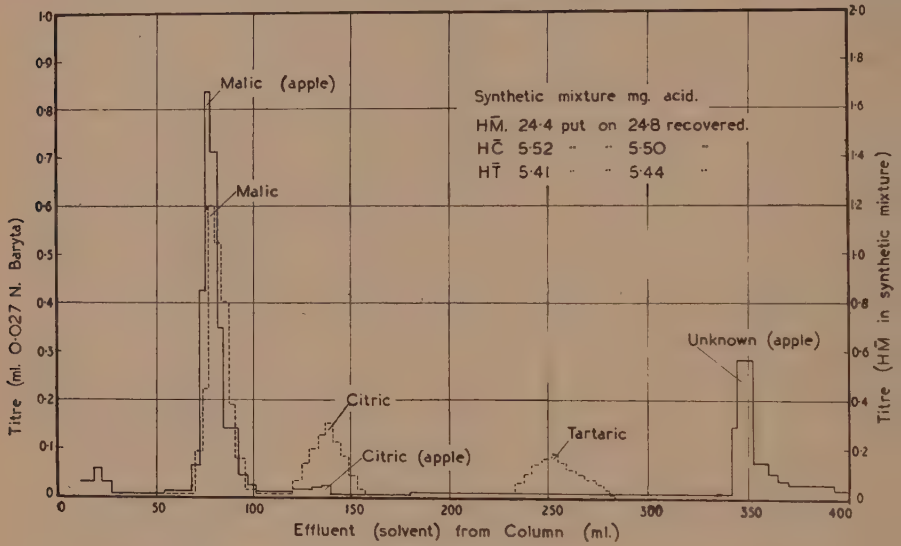
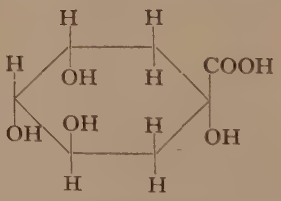


FIG. 1. Chromatogram on Silica Gel of the Organic Acids in an Apple Extract (—) and in a synthetic mixture prepared from authentic specimens of the acids (- - - -).

but it was found that the unknown acid was too insoluble in the organic solvents tried (butanol-chloroform mixtures and methyl-*iso*butyl-ketone) to be prepared satisfactorily in this way. Several grammes of the unknown acid were, however, obtained free from malic and any other acid by means of Deacidite E and Dowex 2 (Davies, Hughes, and Partridge, 1950).

With this supply of the unknown acid it was possible to determine its constitution and to show conclusively that it was *l*-quinic acid, the existence

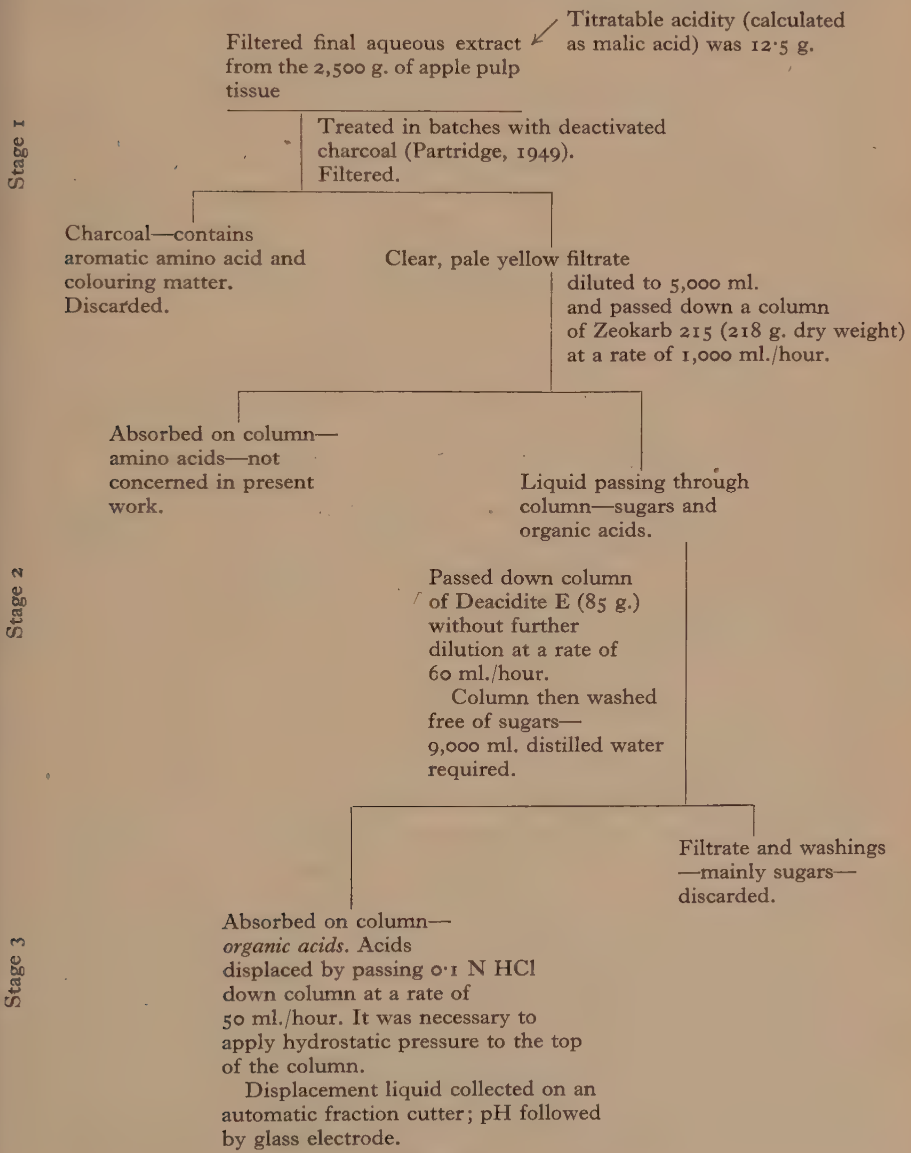


l-quinic acid

of which in the free state in the bark and leaves of certain plants has been known for a considerable time. It is also found as chlorogenic acid in the coffee bean, but, so far as is known, this is the first time that it has been reported in hard fruits.

EXPERIMENTAL

Several hundred fruits of average weight approximately 15 g. were gathered from several Worcester Pearmain apple-trees on 28 June 1950 and were placed in large glass vessels through which was passed a stream of humidified nitrogen in a room at 15° C. The fruits were left in this condition for 20 days. They were then rapidly halved, the seeds removed, and the half apples frozen at −20° C. When frozen, the fruit was peeled at −10° C. and the pulp tissue



Filtered final aqueous extract from the 2,500 g. of apple pulp tissue

Titratable acidity (calculated as malic acid) was 12·5 g.

Treated in batches with deactivated charcoal (Partridge, 1949). Filtered.

Charcoal—contains aromatic amino acid and colouring matter. Discarded.

Clear, pale yellow filtrate diluted to 5,000 ml. and passed down a column of Zeokarb 215 (218 g. dry weight) at a rate of 1,000 ml./hour.

Absorbed on column—amino acids—not concerned in present work.

Liquid passing through column—sugars and organic acids.

Passed down column of Deacidite E (85 g.) without further dilution at a rate of 60 ml./hour.

Column then washed free of sugars—9,000 ml. distilled water required.

Filtrate and washings—mainly sugars—discarded.

Absorbed on column—*organic acids*. Acids displaced by passing 0·1 N HCl down column at a rate of 50 ml./hour. It was necessary to apply hydrostatic pressure to the top of the column.

Displacement liquid collected on an automatic fraction cutter; pH followed by glass electrode.

FIG. 2.

ground to a fine powder also at −10° C. Two thousand five hundred grammes of this powder were plunged in batches of 500 g. into boiling 85 per cent. alcohol. Each batch of tissue, after filtering off the alcohol, was extracted with 85 per cent. alcohol in a vacuum extractor (Hulme and Roach, 1936). All the alcohol extracts were combined and the alcohol removed by evaporation under diminished pressure in a water-bath held at 45° C. The resulting aqueous extract was filtered through an asbestos pad and the pale green opalescent filtrate treated according to the scheme shown in Fig. 2.

The following points should be noted concerning the various stages in the sequence of separations:

Stage 1 (charcoal treatment). Tests showed that the charcoal did not remove any of the titratable acidity.

Stage 2. The ion-exchange columns used had a diameter to length ratio of approximately 1 to 15. It is essential to wash the Deacidite column until a quantitative micro-estimation on the effluent shows sugar to be completely absent, since traces of sugar militate against subsequent crystallization of the organic acids. The size of the resin particles in the Zeokarb column was 44–72 mesh and in the Deacidite column 50–100 mesh.

Stage 3. The displacement of the organic acids with 0·1 N HCl was continued until the effluent gave a faintly positive test for chloride and fraction-collecting was not commenced until the pH of the effluent (initially 6·8) had fallen to 4·5. At this stage approximately 1,300 ml. had passed from the column and subsequent fractions (each of approximately 14 ml.) were 'spotted-up' on wide filter-paper chromatograms run in butanol-formic acid-water (40 : 10 : 50 v/v). The positions of acids on the completed chromatograms were ascertained by spraying the dried paper with bromophenol blue indicator solution.

The maximum number of acids present in any fraction was found to be two, one of which corresponded with a marker spot of malic acid. The pH of the effluent from the column was taken from time to time and its course is shown in Fig. 3.

On the basis of the chromatograms the various (14 ml.) fractions were combined and treated as follows.

The first 36 fractions (approximately 504 ml.) contained one acid only (at present called 'X') having an approximate R_F value of 0·2, and were all combined and evaporated *in vacuo* to about 60 ml. The pale yellow colour was removed by shaking cold with a little Kahlbaum animal charcoal and the bulk was filtered, evaporated under diminished pressure at 45° C., and finally to constant weight (505 mg.) in a vacuum desiccator. This fraction was chromatographically homogeneous when run both in butanol-formic acid-water (40 : 10 : 50 v/v) and propanol-ammonia-water (60 : 30 : 10 v/v). These solvents have been used throughout the present work; the first is due to Lugg and Overell (1947) and will be designated 'B.F.W.' and the second, designated 'P.A.W.', to Hanes and Isherwood (1949).

Fractions 37–44 (approximately 136 ml.). Owing to the dilution at which the 'test' chromatograms were carried out there was some doubt as to which of these fractions contained both 'X' and malic acid, the identity of the latter at this stage being indicated only by comparison with marker spots of pure malic acid. On evaporation, treatment with charcoal, and final evaporation these combined fractions were proved from chromatograms to contain 'X' and malic in approximately equal quantities. The weight of the vacuum-dried crystals was 400 mg.

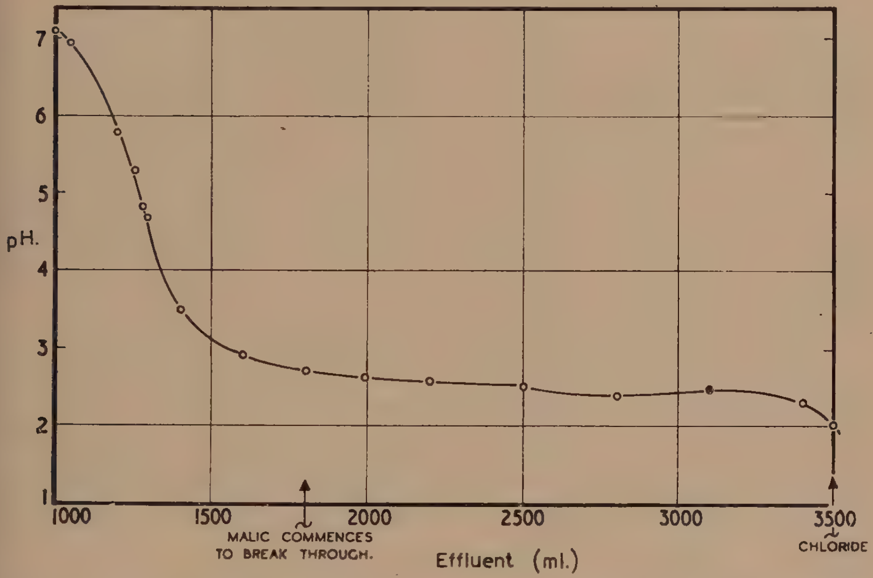


FIG. 3. The pH of Effluents from Deacidite E during displacement of Apple Acids with 0·1 N HCl.

Fractions 45–152 (approximately 1,500 ml.). These combined fractions contained all of the remaining 'X' acid together with much malic acid. The subsequent treatment of these combined fractions will be described later.

Fractions 153–to positive chloride. Chromatograms showed that these fractions only contained acid giving the malic acid spot. They were, therefore, discarded.

Further treatment of combined fractions 45–152

Preliminary experiments had shown that a much sharper separation of 'X' acid and malic acid could be obtained with columns of Dowex 2 than with columns of Deacidite E. The primary separation was not made on the former resin because it is not readily available in this country and requires careful preparation. Scrupulous elimination of CO_2 by means of suitable soda-lime traps is essential during the preparation of the columns, since any CO_2 absorbed on a Dowex 2 column is evolved when acids stronger than H_2CO_3

are passed down the column, which then develops cracks while the edges of the acid bands become very irregular.

The combined fractions (45–152) were evaporated to about 700 ml. Tests showed the evaporate to be completely free from sugars and from any substances reducing silver nitrate. Since acids at a strength greater than about 0·2 N tend to attack the resin, the liquid was diluted to 1,000 ml. before passing down the Dowex 2 column (40 g.; 120–180 mesh) at a rate of approximately 50 ml./hour.

After washing the column with about 500 ml. of CO_2-free water, the acids were displaced with 0·1 N HCl until chloride broke through. Fractions of about 20 ml. each were collected and each fraction was tested on paper chromatograms run in B.F.W. The pH of the effluent was again followed, but the pKs of 'X' and of malic acid are so close that the points of separation cannot be ascertained accurately by following pH changes alone (see Fig. 3).

Fractions 1–20 (approximately 520 ml.) proved, from the chromatograms, to contain 'X' acid only. They were, therefore, combined and decolorized by shaking cold with charcoal. On final evaporation to dryness *in vacuo* the crystals obtained weighed 3·0 g.

Fractions 21–38 (approximately 400 ml.) contained both 'X' acid and malic acid, the former decreasing and the latter increasing progressively. By a second fractionation on Dowex 2, &c., a further 1 g. of 'X' acid was obtained from these free from malic acid (as tested by chromatogram).

Fractions 39–51. Chromatograms indicated that these fractions contained malic acid only. No attempt was made to recover malic acid quantitatively from these combined fractions, but approximately 1 g. of crystals was obtained for detailed analysis (see below).

The various preparations of 'X' acid described above (about 4·6 g. in all) were combined and redissolved in water, the solution being rendered completely colourless by treatment with animal charcoal in the cold and evaporated to a thick syrup. Finally, the syrup was evaporated in a vacuum desiccator over P_2O_5 for several days to yield pure white, almost wax-like, crystals which lost no further weight on further treatment *in vacuo* and were subsequently analysed and identified as quinic acid (see below).

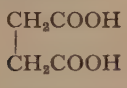
Although the whole of the quinic acid, which must have amounted approximately to 5 g., was not recovered (losses occurred in liquid used for chromatograms and pH measurements and through incomplete recovery from final mixed fractions), some conclusions as to the relative amounts of quinic and malic acids present in the original apple material can legitimately be drawn.

From the equivalent weights of the two acids, 5 g. of quinic acid is equivalent to 1·75 g. of malic acid and, since the titratable acidity of the original extract of the apple tissue was 12·5 g., calculated as malic acid, and no other acids are present in any quantity, the amounts of malic acid and quinic acid were 10·75 and 5 g., i.e. there was about half as much quinic acid as malic acid in the original apple.

Characterization of quinic acid from the apple

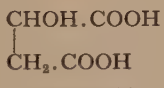
(*a*) *By means of paper chromatography.* The R_F value obtained with the solvent mixture B.F.W., after 24 hours' equilibration with the solvent-poor phase, followed by running by downward flow with the solvent-rich phase using a carefully sealed glass tank in constant temperature room at 20° C., was 0·18 (corresponding R_M value $= +0{\cdot}66$). Under less stringent conditions R_F values as high as 0·22 have been obtained.

Now quinic acid gives strongly positive citrazinic acid and umbelliferone tests (see later), the former being regarded as 'specific' for citric and aconitic acids. This led Hulme and Swain (1951) to examine the chromatographic behaviour of 'X' acid in relation to that of citric acid. Bate-Smith and Westall (1950) have shown that, in several series of polyhydroxy compounds, the nature of which is not important to the present argument, the addition of each OH group decreases the R_M value of the compounds by an approximately constant amount. In the series succinic, malic, and tartaric acids the present author finds that ΔR_M for the OH group is approximately 0·43 in B.F.W. at 20° C.; similarly the ΔR_M value for the OH group in the series tricarballylic, citric, and 'X' acid is approximately 0·48. The evidence up to this point is, therefore, entirely in favour of the suggestion put forward by Hulme and Swain. When, however, these three acids are run in P.A.W. it is clear that 'X' acid cannot in fact belong to the same series as tricarballylic and citric since the R_M's of the three acids are 0·531, 0·695, and *0·115* respectively. Furthermore, in neutral or acid solution the 'X' acid, whether 2 : 3- or 2 : 4-dihydroxy tricarballylic acid, would exist largely in the form of a γ-lactone, which would therefore not fit into the position of the free acid on chromatograms run in B.F.W. The identification of 'X' acid as quinic acid, which, like dihydroxytricarballylic acid, has a total of five hydroxyl plus carboxyl groups, provides a satisfactory explanation of all the chromatographic observations.



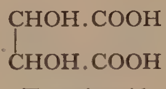
$$\begin{array}{l} CH_2COOH \\ | \\ CH_2COOH \end{array}$$

Succinic acid



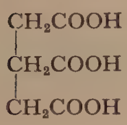
$$\begin{array}{l} CHOH.COOH \\ | \\ CH_2.COOH \end{array}$$

Malic acid



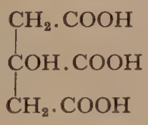
$$\begin{array}{l} CHOH.COOH \\ | \\ CHOH.COOH \end{array}$$

Tartaric acid



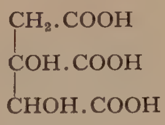
$$\begin{array}{l} CH_2COOH \\ | \\ CH_2COOH \\ | \\ CH_2COOH \end{array}$$

Tricarballylic acid



$$\begin{array}{l} CH_2.COOH \\ | \\ COH.COOH \\ | \\ CH_2.COOH \end{array}$$

Citric acid



$$\begin{array}{l} CH_2.COOH \\ | \\ COH.COOH \\ | \\ CHOH.COOH \end{array}$$

2:3-dihydroxytricarballylic
(hydroxycitric) acid.

In B.F.W., ΔR_M for the OH group in the series succinic, malic, and tartaric acids is 0·43 and for tricarballylic, citric, and quinic, 0·48. In P.A.W., ΔR_M for the OH group in the series succinic, malic, and tartaric is 0·07 and for tricarballylic and citric 0·16. In P.A.W. all five acids fall between the R_F values 0·15 and 0·34. In other words, the OH group is highly functional in

B.F.W. and relatively slightly functional in P.A.W. (where the acids are in the form of the ammonium salts).

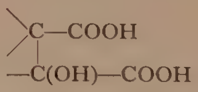
If we consider the —COOH group, ΔR_M in B.F.W. as between succinic and tricarballylic is 0·10, and between malic and citric 0·17. In P.A.W., however, ΔR_M for the two pairs is 0·31 and 0·36 respectively. Thus it seems that the —COOH group is relatively slightly functional in B.F.W. but very much more functional (as —$COONH_4$) in P.A.W.

Although the above suggestions need careful testing with several series of suitable acids before any generalization can be made, they do provide some explanation of the fact that quinic acid has the R_M expected for dihydroxytricarballylic acid in B.F.W., i.e. it has an R_M value greater by the equivalent R_M of one OH group than citric acid, irrespective of the fact that it has two fewer COOH groups and a different configuration from that of tricarballylic and citric acids. The difference of 1 C (tricarballylic, &c., have 6 carbons and quinic has 7) would not be expected to have a very significant effect here.

The high R_F value of quinic acid (one COOH group) in P.A.W. in relation to tricarballylic and citric acids (three COOH groups) is accounted for by the highly functional effect of the —COOH group in this solvent system.

The chromatographic proof of identity of the quinic acid from the apple is provided in Figs. 4 and 5. Fig. 4 is a photograph of a chromatogram in B.F.W. on which three spots were run side by side; one contained quinic acid from the apple, one a recrystallized sample of quinic acid obtained commercially, and the third a mixture of the two. A chromatogram of the same three samples run in P.A.W. is shown in Fig. 5.

(*b*) *By chemical methods. Qualitative tests.* (i) Quinic acid from the apple and the commercial sample gave a strongly positive reaction to the citrazinic acid test (Feigl, 1947*a*). Both samples of the acid also give the strong blue fluorescence in ultra-violet light characteristic of umbelliferones when treated with concentrated sulphuric acid and resorcinol according to the method of Feigl (1947*b*). This reaction is given by acids containing the following grouping



It is suggested that the positive reaction given by quinic acid to these tests is due to the oxidative opening of the ring and the loss of carbon atom 4 to form citric acid. Support is lent to this supposition by the action of H_2O_2 on quinic acid reported below.

(ii) The sample of quinic acid from the apple gave the iodoform test as described for the acid by Struffmann (1927). (iii) *The melting-point* of the acid was 159–160° C. (uncorrected). Commercial *l*-quinic acid had a melting-point of 162° C. and a mixture of this preparation with the one from apple had a melting-point of 161·5° C. (iv) *Optical rotation.* In a 4 per cent. solution the $[\alpha]_D^{21}$ of the apple preparation was $-41°$; the value of *l*-quinic acid given in the literature (see Pigman and Goepp, 1948) is $[\alpha]_D^{20} = -44°$. (v) *Benzoylation*

FIG. 4. Chromatogram of Quinic Acid from the Apple (X) and of a Commercial Sample (Q) (separately and mixed) run in Propanol-Ammonia-Water. (See text.)

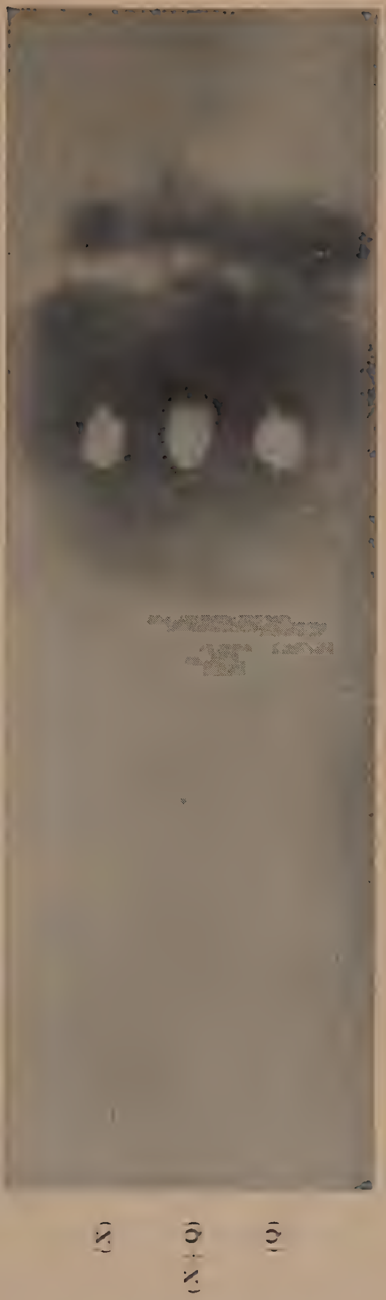
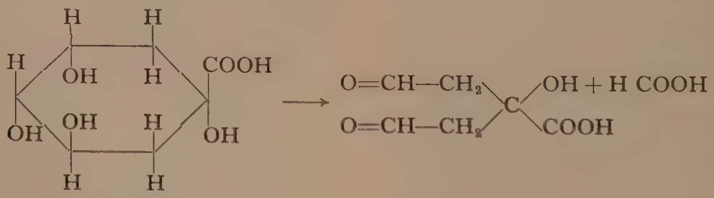


FIG. 5. Chromatogram of Quinic Acid from the Apple (X) and of a Commercial Sample (Q) (separately and mixed) run in Butanol-Formic Acid-Water. (See text.)

under Schotten-Bauman conditions gave a derivative in good yield having a melting-point of 130° C. On analysis this compound contained 67·3 per cent. carbon and 5·0 per cent. hydrogen (required for tetrabenzoate of quinic acid: C = 69·1%, H = 4·6%; for tribenzoate of quinic acid: C = 66·7%, H = 4·7%). (iv) A *p*-bromophenacyl ester of the acid was obtained which had a melting-point of 125° C. (vii) With *p*-nitrobenzyl bromide a derivative was obtained having a melting-point of 74–79° C. The yield of this compound was, however, poor.

Quantitative analysis. (i) The molecular weight of the acid carried out by the method of Barger (1904) using pure glucose as reference compound was 195. (ii) The equivalent weight from titration against standard baryta was 191·6. (iii) The carbon and hydrogen contents of the preparation were 43·1 and 6·42 per cent. respectively. Calculated for $C_7H_{12}O_6$, C = 43·7, H = 6·25; molecular weight 192; equivalent weight as monobasic acid, 192. (iv) After 4 hours' hydrogenation (Adam's catalyst) no evidence of unsaturated groups was found. (v) *Acetylation.* Using the method of Ogg, Porter, and Willits (1945) as adapted by Siggia (1949), the average of two determinations gave the —OH content of the acid as 32·8 per cent. The theoretical value of 3 OH groups would be 26·6 per cent. and for 4 OH groups 35·4 per cent. According to Erwig and Koenigs (1889), acetylation of quinic acid yields triacetyl quinide (lactone). The present results suggest that during acetylation partial lactone formation only took place since the amount of acetic anhydride used up corresponded more nearly to the formation of a tetraacetyl than a triacetyl derivative.

Reaction with periodic acid. According to Fischer and Dangschat (1934) periodic acid oxidizes quinic acid to one molecule of formic acid and one molecule of a dialdehyde as follows.



l-quinic acid

On this basis one molecule of quinic acid should yield 2 COOH groups on treatment with periodate. Portions of quinic acid from the apple were treated with sodium metaperiodate for 20 minutes at 100° C., followed by destruction of the excess periodate with ethylene glycol and titration with standard sodium hydroxide according to the method of Hirst and Jones (1949) Duplicate determinations carried out using different amounts of quinic acid gave results which agreed closely with one another and gave an average of 91·5 per cent. of the theoretical acid (COOH groups) required by the above reaction. Qualitative tests with 2 : 4-dinitrophenylhydrazine confirmed the presence of a carbonyl compound in the reaction liquid.

A quantitative determination was made of the amount of periodic acid converted to iodic acid during reaction with quinic acid from the apple, using the method of Pohle, Mehlenbacher, and Cook as adapted by Siggia (1949). The method employs thiosulphate titration of the iodine liberated in the reaction KIO_4+KI (blank estimation), and the iodine liberated in the reaction between KIO_4, KIO_3, and KI after the oxidation of quinic acid. The yield of iodine in duplicate estimations using an excess of HIO_4 (low results were obtained otherwise) was 96 and 98 per cent. respectively of the theoretical amount for quinic acid based on the reaction above (dialdehyde and formic acid). *The titration curve* obtained for the sample of quinic acid from the apple is shown in Fig. 6. To obtain the curve, 63·8 mg. of quinic acid in 6 ml. of CO_2-free water were titrated with 0·212 N baryta using a micro-burette; pH was measured by means of a glass electrode and the system was protected from CO_2 by soda-lime traps.

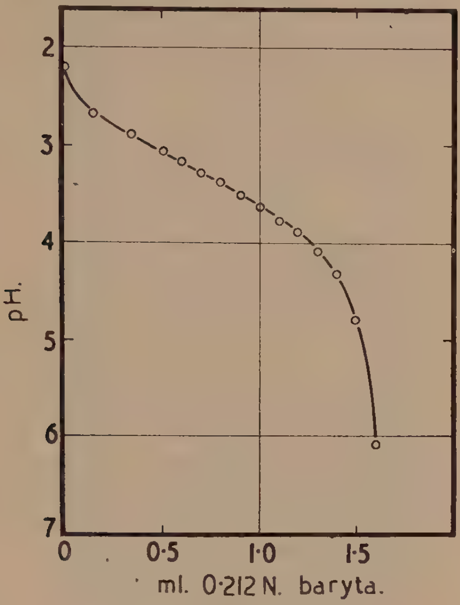


FIG. 6. Titration Curve of Quinic Acid from the Apple.

Oxidation of quinic acid by hydrogen peroxide

The positive reaction of quinic acid to tests designed to detect citric acid has already been mentioned. It was thought possible that mild oxidation of quinic acid by hydrogen peroxide might give products readily detectable by paper chromatography. On refluxing approximately 20 mg. of quinic acid from the apple with 1 ml. of H_2O_2 (100 vol., M.A.R. grade) for periods of from 5 to 120 minutes and chromatographing the resultant solution in B.F.W., it was found that, as oxidation proceeded, the spot on the chromatogram corresponding to quinic acid disappeared and several spots more or less equidistant from one another appeared, all having higher R_F values than quinic acid. Finally, two spots remained. The larger spot on chromatograms run in both B.F.W. and P.A.W. corresponded exactly with the position of marker spots of citric acid. The acid represented by the second spot travelled much faster in both B.F.W. ($R_F = 0{\cdot}66$) and P.A.W. ($R_F = 0{\cdot}31$) than citric acid. When citric acid itself was treated with H_2O_2, and the resultant liquid chromatographed, the stages between citric acid and this fast moving acid, which it has not yet been possible to identify, were reproduced exactly. The chromatograms obtained with quinic acid from the apple and with the commercial sample of the acid after heating with H_2O_2 for 40 and 80 minutes are shown in Fig. 7. These chromatograms (including those in P.A.W. not shown) not only provide reasonable proof that quinic acid may be oxidized to citric acid but give striking support for the identity of the quinic acid from the apple. It would be an interesting exercise to isolate by silica gel chromatography the various stages in the oxidation of quinic and citric acids.

Chemical characterization of l-malic acid from the apple

The sample of the acid corresponding to the position of malic acid in chromatograms (see p. 304, under 'Fractions 39–51') gave the following results: Melting-point, 101° C.; C, 35·7 per cent., H, 4·7 per cent.—the calculated values are 35·8 and 4·5 per cent. respectively. The silver salt contained 61·6 per cent. Ag—calculated for $Ag_2C_4H_4O_5$; Ag = 62·0 per cent., 39·4 mg. of the free acid +272·4 mg. ammonium molybdate in 25 ml. water gave $[\alpha]_D^{20}+569°$; McKenzie and Plenderleith (1923) record $[\alpha]_D^{15} = +568°$ under similar conditions. The melting-point of the *p*-nitrobenzyl ester was 124·5° C. The equivalent weight of the acid was 66·6.

DISCUSSION

The occurrence of relatively large amounts of quinic acid in young actively metabolizing apple fruits poses the question of its role in the metabolism of these fruits. Furthermore, this question has wider implications because not only is the acid present in these young fruits stored in air as well as nitrogen but, from chromatographic evidence, it is also present in freshly gathered young 'Conference' pears and in plums and peaches (Hulme and Swain, 1951). It would appear from general evidence that the amount of quinic acid is considerably less in mature apples and pears but, until the completion of ontogenetic studies of its quantitative distribution in these fruits now in progress, this point is in doubt. Over a period of years the presence of quinic acid (free or combined with caffeic acid as chlorogenic acid) and the closely allied shikimic acid have been reported in a wide variety of plant materials, e.g. green coffee beans, cinchona bark, tops of whortleberries, leaves of the mountain cranberry, and fruits of the star anise (*Illicium verum*). Oparin (1927) even suggested that chlorogenic acid might act as a respiratory chromogen undergoing oxidation by atmospheric air to a green pigment capable of acting as a hydrogen acceptor.

In spite of the widespread occurrence of quinic acid in the plant world two

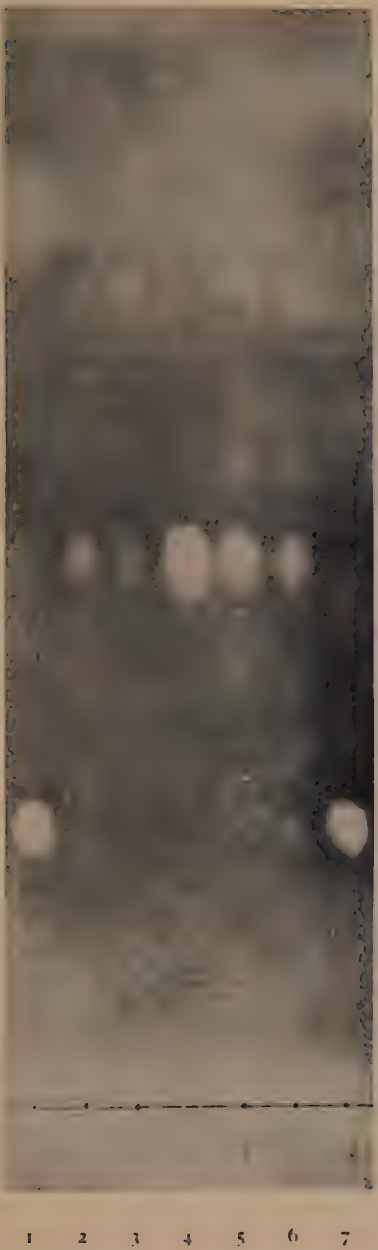


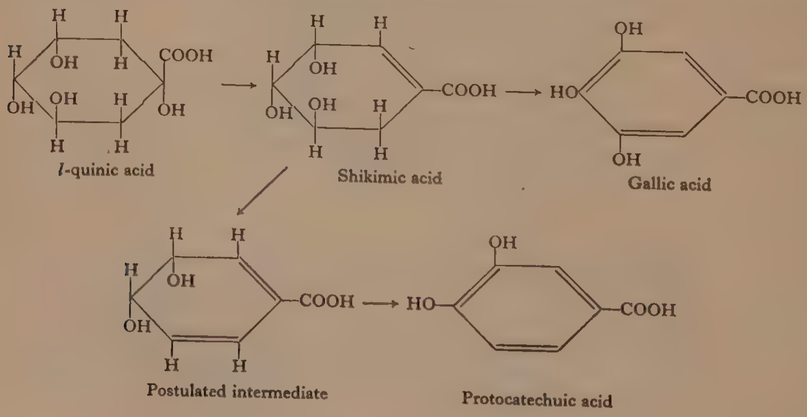
FIG. 7. Chromatogram of the products of the oxidation of quinic acid by hot hydrogen peroxide. 1, Apple quinic + H_2O_2 before heating. 2, Ditto after 40 mins. at 100° C. 3, Ditto after 80 mins. at 100° C. 4, Citric acid. 5, Commercial quinic after 80 mins. at 100° C. 6, Ditto after 40 mins. 7, Ditto before heating.

recent reviews, devoted wholly or partly to the metabolism of organic acids in plants (Thimann and Bonner, 1950; McKee, 1949), do not mention the acid although the presence is mentioned, in various circumstances, of 'unknown acids'. For example, Ruben, Hassid, and Kamen (1940) showed that during the fixation of labelled CO_2 by *Chlorella pyrenoidosa* much of the labelled C was present as carboxylic groups not due to ascorbic, citric, malic, fumaric, maleic, succinic, oxalic, or tartaric acids. Possibly this hiatus is due to the fact that, until quite recently, practical studies in the 'aliphatic' and the 'aromatic' metabolism of plants have tended to be pursued along quite separate and distinct lines, e.g. 'organic acid metabolism' has really meant 'aliphatic organic acid metabolism'. This restricted outlook has not been so noticeable in theoretical studies, and Hall (1937) in a stimulating, if highly speculative, review of relationships in phytochemistry placed quinic acid in a key position as the link between the aliphatic and aromatic compounds in plants. The views of Hall, purely speculative though they may be, will be referred to again later; meanwhile it is profitable to consider reactions involving quinic acid which are definitely known to occur *in vivo* and *in vitro*.

In vitro *reactions of quinic acid*

Fischer and Dangschat (1934, 1935) succeeded in oxidizing quinic, shikimic, and dihydroshikimic acids to citric, aconitic, and tricarballylic acids respectively by means of periodate followed by bromine. These workers (1938) also succeeded in converting quinic to shikimic acid. Evidence for the oxidation of quinic acid to citric by means of hydrogen peroxide is provided in the present paper.

The aromatization of quinic acid has been known since the middle of the nineteenth century, e.g. Lautemann (1863) heated quinic acid with hydriodic acid and obtained benzoic acid; Hesse (1880) used hydrochloric acid to produce *p*-hydroxybenzoic acid. Protocatechuic acid was obtained by Fittig and Macalpine (1873) by treatment of quinic acid with aqueous bromine. Several authors have suggested that gallic acid might be produced from quinic acid, and Gisvold and Rogers (1938) suggest the following sequence of events:

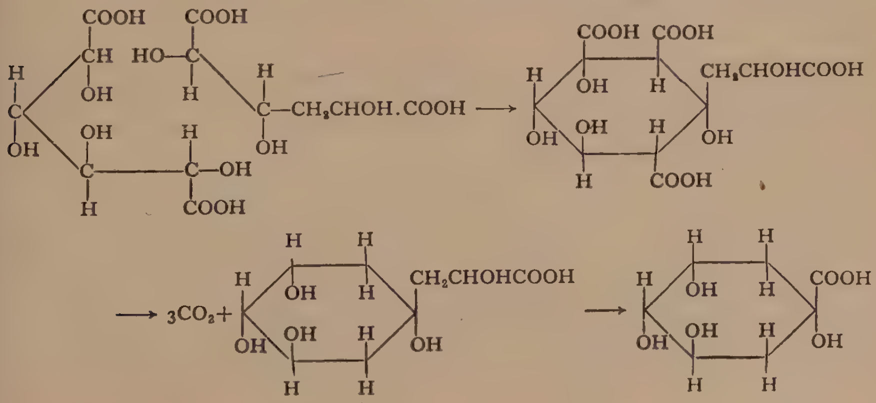


Fischer and Dangschat (1937) succeeded in converting shikimic acid (and therefore quinic acid also) into 2-desoxy-D-gluconic acid, suggesting a possible biogenetic relationship between quinic acid and D-glucose.

In vivo *reactions of quinic acid*

There appears to be, as yet, no evidence of the conversion of quinic to citric acid and vice versa in plants, but this may be merely a reflection of the almost complete absence of metabolic studies involving quinic acid. Hall (loc. cit.) suggests that citric and quinic acids are not generally found in the same plants and that the two acids rather have a common precursor. Until more is known about the distribution of quinic acid it is impossible to generalize on its presence or absence in citric acid-containing plants.

Hook (*vide* A. Boldt, 1937) found both quinic and shikimic acids in the carpels of *Illicium verum*, but two results reported by Gordon, Hoskins, and Mitchell (1950), when taken together, suggest that quinic acid may not be the precursor of shikimic acid, at least in the lower forms of plant life. These authors mention the work of Davis in which shikimic but not quinic acid was found to be capable of replacing tyrosine, phenyl alanine, &c., in promoting the growth of a mutant strain of *E. coli*. Gordon, Hoskins, and Mitchell themselves found that quinic but not shikimic (and other benzenoid compounds) would promote the growth of *Neurospora* mutant C-86. This work does, however, show that bacteria and fungi can bring about the aromatization of quinic acid, a fact also shown for moulds by Butkewitsch (1924). These results suggest rather that quinic and shikimic acids have a common precursor. In this connexion, Hall (loc. cit.) suggests that the biogenetic origin of compounds of the type of quinic acid is by way of a condensation of one molecule of metasaccharonic acid and one of a saccharic acid. Kiliani and Scheibler (1889) obtained the metasaccharonic acid 3-desoxy-D-galactonic acid from *d*-quercitol which is allied structurally, but not configurationally, to shikimic acid. The metasaccharonic acid postulated by Hall agrees configurationally with the desoxygluconic acid obtained by Fischer and Dangschat from quinic acid (see above). Hall's views suggest the following synthesis of quinic acid in the plant:

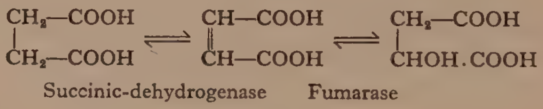


The fact that metasaccharonic acids do not naturally arise by the oxidation of simple sugars Hall does not consider of great importance since he regards such compounds as produced directly by degradation of a much larger condensed (polysaccharide) molecule.

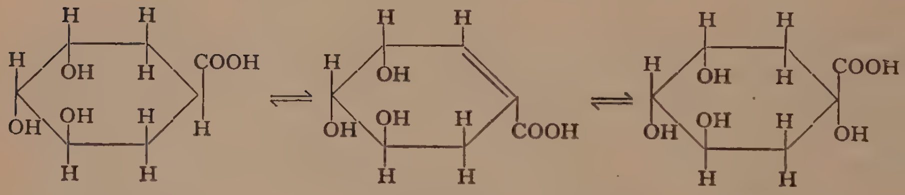
Whatever the method of synthesis of quinic acid in the plant the subsequent desaturation of its ring to give benzenoid compounds seems extremely probable.

The foregoing facts provide considerable presumptive evidence for the hypothesis that quinic acid provides one link between what might be called the aliphatic and aromatic metabolisms of the plant.

It was further suggested to the author by Dr. L. W. Mapson that the trio of alicyclic acids might conceivable form part of a dehydrogenase system analogous to the succinic-fumaric-malic system, as follows:



$$\begin{array}{l} \mathrm{CH_2{-}COOH} \\ | \\ \mathrm{CH_2{-}COOH} \end{array} \rightleftharpoons \begin{array}{l} \mathrm{CH{-}COOH} \\ \| \\ \mathrm{CH{-}COOH} \end{array} \rightleftharpoons \begin{array}{l} \mathrm{CH_2{-}COOH} \\ | \\ \mathrm{CHOH.COOH} \end{array}$$



These speculations serve to suggest several experimental lines of attack on the physiological problem presented by the presence of quinic acid in fruits. The circumstances of its occurrence and the implications of its structure are too significant for its function to remain unexplored.

I am greatly indebted to Drs. E. C. Bate-Smith and T. Swain for valuable discussions during the course of the identification of quinic acid and to Dr. L. W. Mapson during discussions on the possible function of the acid in the fruit.

During the course of the characterization of the acid, Dr. A. E. Bradfield put forward valuable suggestions and it was he who, when the end was in sight, suggested that the acid, in spite of some evidence apparently to the contrary, might well be quinic acid.

My thanks are due to the Director of the Chemical Research Laboratory, in whose micro-analytical laboratory the carbon and hydrogen determinations were carried out, and to Dr. A. F. Colson of Imperial Chemical Industries (Alkali Division) Research Department for testing quantitatively for unsaturated groups before the identity of the quinic acid had been established.

Mr. W. Arthington provided experimental assistance during the course of the work.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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The Path of Carbon in Photosynthesis

XII. SOME TEMPERATURE EFFECTS[1]

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SUMMARY

The photosynthetic assimilation of radioactive carbon dioxide for 2-minute periods by *Scenedesmus* has been studied at temperatures ranging from 25° to 44° C. All labelled intermediates cease to be formed at about 45° C. With rising temperature, the radioactivity reaching the sugar phosphate reservoirs decreases regularly while there is a sharp maximum in sucrose at 37° C., and a less pronounced one in malic and aspartic acids above 40° C. A tentative interpretation of these effects is offered.

INTRODUCTION

A CHANGE in temperature, especially near that at which photosynthesis ceases to take place, is likely to exert selective effects upon the rates of the various enzymatic reactions involved in carbon-dioxide assimilation as revealed by the pattern of the labelled intermediates (Calvin and Benson, 1948; Benson and Calvin, 1950; Calvin, Bassham, and Benson, 1950). In an attempt to bring out such effects, the photosynthetic fixation of radioactive carbon dioxide by *Scenedesmus* was studied at temperatures ranging from 25 to 44° C. Experiments of this kind suffer from one of two possible limitations: (*a*) a disturbance of the steady state by the transition phenomena arising from a sudden temperature change, or (*b*) the long-range changes in enzyme constitution through which growing cells adapt themselves to a new temperature. Since the latter effect would more likely tend to restore a normal metabolic balance, brief thermal shocks and photosynthetic periods were used in this preliminary investigation.

EXPERIMENTAL

For a series of experiments, a 1-day growth from 1 litre of a continuous culture of *Scenedesmus* (Benson, Calvin, *et al.*, 1949) was freed from the culture medium by centrifugation and resuspended in M/3000 phosphate buffer (pH 6·7) at a concentration of 1 g. wet-packed cells in 100 c.c. A stream of air was passed during 1 hour through this cell suspension kept at room temperature in subdued daylight. The reaction vessel was a 250-c.c. Erlenmeyer flask in which a 20-c.c. aliquot of cell suspension built a layer which was illuminated from underneath at 1,200-foot candles from a bank of fluorescent lights. These lights were placed under a glass dish filled with water in which the reaction vessel was immersed. The temperature was read

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[1] The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

[2] Guggenheim Fellow 1949–50, on leave from Laval University, Quebec, Canada.

Journ. of Experimental Botany, Vol. II, No. 6, pp. 316–320, September 1951.

on a thermometer dipping into the layer of algae and held in position by a rubber stopper. In an experiment the cells were first adapted to light of 1,200 foot candles during a period of 10 minutes. Hot water was then added to the water in the dish and the temperature in the cell layer rose in about 2 minutes to a final value where it could be kept constant within 0·5° C. At this point 70 μl. of a radioactive sodium bicarbonate solution (11 microcuries, 0·001 millimoles) were added, the flask stoppered, and the cells allowed to photosynthesize during 2 minutes, after which they were killed rapidly in boiling ethanol. During the whole adaptation and photosynthesis periods the cell suspension was kept in motion by gentle horizontal shaking. The extract in 80 per cent. ethanol was analysed by the radiochromatographic method (Benson, *et al.*, 1950).

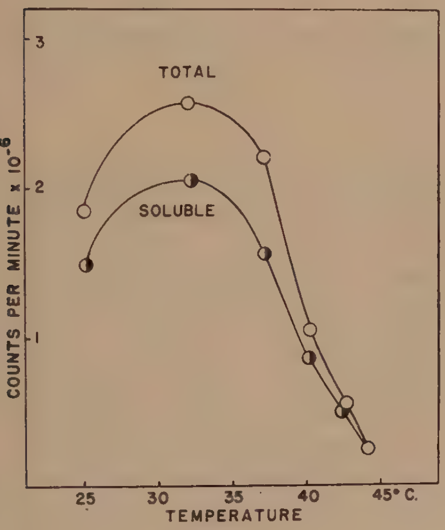

FIG. 1.

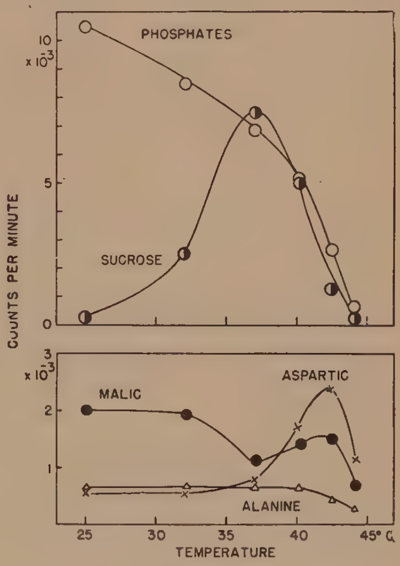

FIG. 2.

FIG. 1. Temperature dependence of the rate of fixation of carbon dioxide by *Scenedesmus* in 2-minute photosynthesis at 1,200 foot candles. Radioactivity in millions of counts per minute per gram cells: Total (○), soluble in 80% ethanol (◑).

FIG. 2. Radioactivity in some soluble intermediates, in counts per minute per 50-mg. cells, on paper chromatograms. Lower section on enlarged scale.

RESULTS

Preliminary trials indicated a large increase in the percentage of labelled sucrose with rising temperatures for short experiments. The experiments described here were carried out at 25, 32, 37, 40, 42·5, and 44° C. within a period of about 2 hours and with samples from the same growth of algae. Fig. 1 shows the total radioactivity fixed and the part which is soluble in 80 per cent. ethanol. The radioactivities fixed during 2 minutes in the various intermediates were obtained by counting the corresponding spots on the paper chromatograms. Some of the data are shown in Fig. 2. The main effects of raising the temperature are the gradual decrease in the labelled phosphates, the surge of labelled sucrose up to 37° C., and the remarkable convergence of all the curves towards the same limiting temperature of about 45° C. The sum of the radioactivities in glycine and serine (not shown on the diagram) is very nearly equal to the radioactivity in alanine and follows the same trend, i.e. remains constant almost up to the limiting temperature. On the other hand, the four-carbon compounds malic and aspartic acids show a significant increase near 43° C., just below the upper limit.

In a few experiments designed to test the reversibility of these effects the algae were first subjected to the higher temperature for a period of 2 minutes and rapidly cooled down to room temperature for a 2-minute photosynthetic fixation of $C^{14}O_2$. When the results of such 2-minute shocks at 39° and 43° C. were compared with those of a blank experiment at 25° C., the only large difference was in the percentage of labelled sucrose, which was intermediate between that in the low and in the high temperature experiments. It thus seemed that no permanent change had taken place during the brief thermal shock.

DISCUSSION

The simultaneous decrease in rate of formation of all the labelled intermediates at about 45° C. suggests that a key step is blocked at that temperature, possibly by the heat denaturation of an enzyme or by the alteration of some structure in the cell. The point of breakdown must be located either at the earliest stage of carbon-dioxide fixation or at an anterior stage connected with the photolysis of water by the chloroplasts, which are known to become rapidly inactivated in that temperature region (Holt and French, 1946; Arnon and Whatley, 1949). If the latter were the case, one could expect enhanced dark fixation to occur above 45° C. after pre-illumination at room temperature.

A tentative explanation of the effects shown in Fig. 2 can be offered on the basis of a reaction scheme of the type of that which has been proposed earlier (Calvin, 1949). In a regenerative cycle comprising two-, three-, and four-carbon intermediates a reversed glycolytic sequence branches off from a three-carbon intermediate and leads to sucrose, while another branch leads from a four-carbon intermediate to malic and aspartic acids (Bassham, Benson, and Calvin, 1950). As the temperature rises from 25° to 37° C., the passage from the hexose phosphates to free sucrose is greatly accelerated, so that more of the assimilated carbon reaches sucrose and less accumulates in the phosphates. Above 37° C., the access to the glycolytic branch becomes more difficult, presumably as the result of heat deactivation of an enzyme, so that the radioactivity decreases simultaneously both in the phosphates and in sucrose. However, this decrease benefits the competing malic-aspartic reservoir, into which C^{14} now accumulates more rapidly, until a temperature is reached at which C^{14} ceases to be incorporated into the regenerative cycle. The fact that the rate of fixation into alanine seems independent of temperature between 25° and 40° C. suggests that the rate of an important step is controlled by diffusion.

An attempt to apply the Arrhenius equation to the individual variations led to the results shown in Fig. 3, which permit a rough evaluation of the temperature increments. The slopes for the phosphates and for sucrose above 40° C. correspond to energies of -100 to -150 kcal./mol., which are of the right order of magnitude for the activation energies of enzyme inactivation. The sucrose curve between 25° and 37° C. yields 50 kcal./mol., possibly the resultant activation energy of a few consecutive anabolic steps. In spite of the complexity of the curves for malic and aspartic acids, the ratio aspartic/malic between 32° and 44° C. shows a constant slope corresponding to an energy of 28 kcal./mol. Assuming that these two acids accumulate outside the regenerative cycle (Bassham, Benson, and Calvin, 1950) and are in equilibrium with each other, one might consider this temperature effect as an equilibrium shift and the above energy as the energy of the corresponding reaction. Although these interpretations are only speculative, the results of these preliminary experiments indicate that some of the intermediate steps in photosynthesis are accessible to thermal analysis.

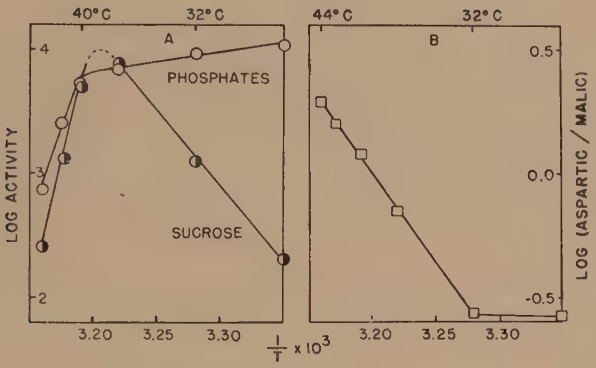

FIG. 3. 1/T v. logarithm of (A) the radioactivities in the phosphates and in sucrose; (B) the ratio of the radioactivities in aspartic and malic acids.

It should be made clear that the rate of appearance of radioactivity in any intermediate depends not only on the rates of formation and utilization of the intermediate but upon the concentration of previous intermediates as well. Consequently, the experimentally observed results are dependent upon the effects of temperature upon the numerous rate constants of this rather complex system.

ACKNOWLEDGEMENTS

I express my thanks to Professor M. Calvin and Dr. A. A. Benson for their continued interest in this work and to the John Simon Guggenheim Memorial Foundation for a Fellowship.

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Observations with the Electron Microscope on a Species of *Saprolegnia*

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WITH PLATES 1–8

SUMMARY

Electron micrographs are presented to show the morphology of the flagella in each of the two motile phases of *Saprolegnia ferax*, and one visual light photograph of a stained cell of the second stage is added. The front flagellum of both phases is a *Flimmergeissel* with hairs between 2 μ and 3 μ long arranged in two rows and each ending in a thin hair-point. The axis is covered by a wide transparent sheath made of some material which appears to liquefy after death. The hind flagellum, which is devoid of *Flimmer*, is covered by a similar sheath, though there are signs that this is flattened into a fin in the hind flagellum of the second stage. Some very fine short hairs 0·5 μ long are interpreted as a possible internal framework to this fin. The axis of both flagella is fibrillar, but accurate numerical details have not yet been obtained. The skins discarded from cysts have also been examined and shown to be covered with characteristic double-headed hooks, which are strong enough to attach a cyst to passing objects.

HAIRY flagella (*Flimmergeissel*) have been studied by us with the electron microscope in various brown algae (Manton and Clarke, 1950, 1951*b*) and certain flagellates (Manton, 1951), while cilia without hairs have been examined in a fern (Manton and Clarke, 1951*a*), the liverwort *Marchantia* (Manton, 1951) and in the zoospores of the green alga *Ulothrix* (Manton, 1951). In extending the inquiry now to include a fungus, the choice of *Saprolegnia* as a starting-point was a very obvious one since hairs on the front flagella of the zoospores in a number of water moulds have been demonstrated with bacteriological staining and the light microscope by Vlk (1939), Couch (1941), Saksena, Bhargava, and Din Dayal (1943), and perhaps other workers; there are therefore known to be features of interest to investigate from a comparative standpoint.

Among the water moulds the genus *Saprolegnia* seemed particularly suitable for study for a number of reasons. It is easy to catch in the wild, easy to cultivate, and it possesses both motile phases. This phenomenon (diplanetism) by which the asexual zoospore has two periods of motility separated by a period of encystment is characteristic of the Saprolegniales, but in some genera one or other of the motile periods is curtailed or even suppressed. Since it was at least possible that the two motile phases might differ significantly, it was necessary to restrict the inquiry to any one individual species of *Saprolegnia* and to separate it from other species or genera which might be caught at the same time. Crude separation of this type can readily be effected even from a mixed culture by detaching individual sporangia with needles under a dissecting microscope and following the subsequent fate of the zoospores emerging from such a sporangium when placed in a hanging drop of water. In this

Journ. of Experimental Botany, Vol. II, No. 6, pp. 321–331, September 1951.

way the crude characterization of the two motile phases can also be carried out, and the first general views taken with the low power of the electron microscope to show the morphological distinctions between them were from material treated in this way: hence the prevalence of bacteria in some of the pictures.

For observation of the finer details at higher magnifications further purification was carried out to obtain single-spore cultures free from bacteria. The specific identification followed later when old cultures could be induced to form sex organs. These were of the *S. ferax* type, but it will be obvious that the selection of this species for study was in a sense fortuitous since it was merely the first of its kind which happened to come to hand, in which ready induction of zoospores could be achieved in a manner sufficient for the preparation of electron microscope mounts.

Without knowing whether some other species of *Saprolegnia* might have proved easier to handle from the point of view of electron microscopy, it is fair to say that this one presents certain difficulties which we have not previously encountered. In spite of the absence of plastids the spore bodies are too large to become transparent even when flattened and we have not been able to learn anything about their contents. Further, they decompose very readily after killing, but generally in an uninformative manner. Attempts to harden the cilia by chemical treatment have on the whole been unsuccessful, while the application of chemical treatments to the living cell is impeded by the great readiness with which both stages encyst with slight changes of conditions. Finally there is the difficulty of obtaining uniform spores of the right stage in sufficient quantity.

For all these reasons, and in view of the number of different types of cell to be examined, an exhaustive study of every aspect of the material is scarcely possible in the space of one communication. We have therefore concentrated our attention in the first instance on establishing the more obvious morphological facts for the three stages under consideration, leaving certain of the more difficult details for further elucidation on a later occasion.

MATERIAL AND METHODS

Many water moulds can be caught by the very simple procedure of dropping boiled split hemp-seeds into water collected from a pond or well-established aquarium. After a few days at room temperature a mycelium visible to the naked eye develops from the cut surface. In the first instance this is always a mixture of organisms, but purification by subculturing presents no difficulty.

In the present instance the water used was from a laboratory aquarium and the hemp-seeds had been cooked by being sterilized in water in closed tubes in the autoclave. All cultures were kept in the dark in an incubator at 25° C. When the first mixed growth had appeared a small piece of promising mycelium was detached, washed in sterile water, and transferred to a fresh hemp-seed on plain 3 per cent. agar in a Petri dish. After two days a disk of mycelium some 5 cm. in diameter had formed in the agar and a small portion of the rapidly advancing edge was transferred to another fresh hemp-seed in autoclaved water from the aquarium. The growth on this seed proved to be a *Saprolegnia* and it became the parent culture for this study.

The first observations on the morphology of the various stages were made on the products of individual sporangia from this culture which were dissected out under the low power of the microscope and transferred to hanging drops of water. Such isolation of single sporangia proved a very satisfactory means of studying the serial sequence of stages, but bacterial contamination was difficult to prevent and the number of spores produced at any one time was inconveniently limited.

To overcome these difficulties it was necessary to establish cultures originating from a single spore and to multiply these under conditions leading to a mass production of sporangia. These two requirements were met as follows. Single-spore cultures were established by streaking zoospores from the parent culture on to agar and then cutting out an area containing only the mycelium from one recently germinated spore. This was then subcultured on hemp-seed agar made up as follows. A hemp-seed broth was made by boiling 30 g. ground hemp-seeds for 2 hours in 1 litre of tap-water and filtering. This was mixed as required with an equal volume of 3 per cent. agar in distilled water. The agar and broth were separately sterilized in 10-c.c. lots in the autoclave at 20 lb. for 30 minutes and the mixing was done as the plates were poured. Vegetative growth was very vigorous on this medium and, by subculturing, it was easy to obtain bacteria-free inocula from the advancing edge of the mycelium. The clone so isolated was the source of material for the electron microscopy of fine details.

The production of zoospores was, however, inhibited on hemp-seed agar, and both on this and in sterile hemp-seed broth the mycelium tended to grow vegetatively without reproducing except by gemmae cut off from the tips of the hyphae. Reproduction by zoospores was, however, at once stimulated if pieces of well-fed mycelium were transferred to water. The procedure adopted for this was to transfer in the first instance a number of small inocula, each about 3 mm. square, from an agar culture to a broth culture. After 12–24 hours, when each mycelium had grown to a diameter of 2–3 cm., two or three were removed, vigorously washed in several changes of sterile water, and put together into about 5 c.c. of sterile water in a 2-in. Petri dish. Under these conditions, at 25° C. the formation of sporangia takes place rapidly; dehiscence begins after about 4 hours and a dense suspension of zoospores of uniform age and origin results. This method of obtaining a crowded spore suspension closely resembles that advocated by Cotner (1930).

Under uncontrolled conditions the time sequence of subsequent events varies considerably since both the spores and the cysts seem sensitive to a variety of known and unknown factors which include temperature, aeration (which is affected by the depth of available water), nutrients, and the previous history of the culture. Encystment may occur in first-stage swarmers a few minutes after liberation or only after more than an hour and not all the spores in one culture behave alike. For this reason it is desirable to examine first-stage swarmers as soon as possible after liberation. A high proportion of encysted stages is to be expected about 4 hours after spore liberation has begun, but to obtain a concentrated suspension of second-stage swarmers free from admixture with belated first-stage cells required a good deal of experimenting and the method finally adopted was as follows. From a culture prepared as described in the last paragraph the mycelium is removed 4 hours or so after liberation of zoospores has begun, thus preventing the addition of any new first-stage cells. The suspension of motile and encysted cells remaining in the Petri dish is then poured into a narrow tube 1·5 cm. wide, thus increasing the depth of water. The tube is corked up and after being left at room temperature for 8–12 hours (or overnight) a dense suspension of second-stage motile cells without any first-stage cells will be found to have accumulated in the topmost centimetre of water. Samples from this can be pipetted off and used at once, but a sufficient concentration of active cells will remain in a suitable condition for electron microscopy for a further period of 6–7 hours at room temperature.

The production of sex organs, which are required for identification of the species, only occurred when the plant was grown on a solid nutrient substrate in water. The nutrients tried included hemp-seeds, maize, peas, and *Drosophila* grubs on several of which sex organs were obtained under some, though not under all, conditions. The particular requirements for the effective production of sex organs are outside the scope of the present study and need not be further specified. The sex organs obtained, both in the parent culture and in the single-spore cultures derived from it, conformed to the morphological characters of *S. ferax* (Gruith.) Thuret as described by Coker (1923). The oogonia varied from 23 μ to 113 μ in diameter and contained from 1 to about 30 eggs ranging from 17 μ to 23 μ in diameter. Antheridia were rarely seen in any of the cultures.

With regard to the technical methods specially connected with electron microscopy, there is nothing new to report beyond what has already been said in previous papers. The zoospores were killed by about 30 seconds exposure to a saturated atmosphere of osmic vapour, blotted, and dried down on a formvar film. They were shadowed with gold-palladium and examined with the Philips electron microscope. A word of warning should, however, perhaps be given regarding the citation of magnifications. We have not yet succeeded in obtaining a completely trustworthy method of standardizing these to make allowance for fluctuations in the dial readings on the microscope with different voltages and on different occasions. The magnifications indicated on the plates should therefore be regarded as approximately correct but not sufficiently so to permit of very fine measurements.

The staining technique used for the one observation recorded with visual light (Fig. 15) is a modification (cf. p. 330 below) of Kirkpatrick's method published in Mackie and McCartney, 1942.

Morphology of the two motile phases

Some idea of the general appearance of the two motile phases and of cysts can be obtained from Figs. 1–3, Pl. I. The cysts in Fig. 2 are represented by their empty skins, but Figs. 1 and 3 are of dried zoospores from the two motile phases as seen at a very low magnification (× 3,000) in the electron microscope. All three figures are from hanging-drop cultures of isolated sporangia as described on p. 323. The abundance of bacteria visible in Fig. 3 is due to the rapid multiplication of chance contaminants in the hanging drop during the several hours which must elapse for the zoospores to pass from the first stage to the second. In any one culture, with or without bacteria, a very considerable diversity of appearances and behaviour is to be found even when only the products of dehiscence of a single sporangium are present. The cells illustrated have therefore been chosen according to our judgement of what is a fair sample of characteristic cells which will serve to indicate certain commonly found features, but a different result could have been obtained had selection been exercised on some other basis.

First-stage cells, i.e. cells which have just emerged from the sporangium but have not yet encysted, possess two flagella of rather similar length attached to the body in such a way that after death of the cell they commonly point in the same direction (forwards) even though, while swimming, one points forwards and the other backwards (cf. Couch, 1941). The forwardly projecting flagellum is usually a little, though not much, shorter than the other and, as will be shown later, it is a *Flimmergeissel*. The body in many cases tends to be spherical, it is conspicuously so in Fig. 1, but when nutrition of the parent mycelium is improved the spore bodies become larger, in which case they may be oval or slipper shaped with a slight median or submedian constriction, as may be seen in the body of Fig. 4, Pl. II, but the flagella are in that case attached terminally or nearly so and not at the point of constriction.

First-stage swarmers never swim for very long. They may round up and encyst almost at once after liberation or they may swim for over an hour before doing so. They rarely, if ever, swim for more than 1½ hours, under the conditions used by us.

The second-stage swarmers tend to be slightly smaller in body than first-stage cells of the same brood. They are commonly oval in outline (cf. Fig. 3), although they may sometimes round up after death (cf. Fig. 11). In life there is often a slight furrow visible near the point of attachment of the cilia which gives the 'kidney shape' to the whole cell, which has been commented on by visual microscopists, though this is usually lost after death. When dried the outline of the body shows a characteristically undulated edge and its periphery is commonly transparent to the electron beam, especially in the larger cells (cf. Fig. 11). Both these details may perhaps suggest that there is some difference in the state of the surface layers of protoplasm immediately before and immediately after encystment.

The two cilia, in second-stage cells, are always attached laterally in life and at such an angle that commonly one projects forwards and the other backwards in a very conspicuous manner even after death (Fig. 3). Even when this attitude is lost, however, as in the cell of Fig. 11, there is a very constant difference in the relative dimensions of the two flagella in second-stage cells compared with first-stage cells. The actual lengths of the flagella may vary considerably from cell to cell and with the method of drying after they have been killed. There is, however, always a greater disparity between the front and the hind flagella in the second stage compared with the first, the hind flagellum frequently being almost twice the length of the front one, in the second stage.

The swimming motion of second-stage swarmers is almost certainly correlated with this. On a casual inspection they give the impression of swimming faster than first-stage cells and they certainly swim for longer under suitable conditions. We have not attempted to keep individual cells under observation for the very long periods that would be required for accurate timing, but to judge by the behaviour of a mixed suspension the average period of motility of second-stage cells under our conditions cannot be much less than 8 hours and may in certain cases be longer. At the end of this period the cells encyst for the second time, a process which we have not examined in detail, and these cysts normally germinate by means of a tube.

The cysts

The external signs of encystment, visible with the light microscope at the end of the first motile phase, are that a zoospore suddenly stops swimming and rounds up. The membrane which then covers it is very thin and is easier to see when the cell again emerges from it for the second motile phase, for it is then discarded empty and can be readily picked up (as in Fig. 2, Pl. I). What happens to the flagella during the act of encystment we have been unable to determine, though we have no reason to think that they are merely thrown off since whole detached cilia are never easy to find and they are certainly not more numerous at the end of the first motile period than at other times.

The duration of encystment is somewhat uncertain since it is difficult to estimate it accurately in a mixed suspension which can never be entirely synchronized. It also certainly varies within wide limits and it can be strongly influenced by environmental conditions, though we have not examined this in any detail. In some cases we have reason to think that a first-stage cyst can germinate by means of a tube as if it were a second-stage cyst, as described by Lechmere (1910). The usual behaviour under our conditions is, however, that after a dormancy of several hours the cyst membrane is discarded and the second-stage swarmer emerges.

The discarded cyst membranes, after emergence of the second-stage swarmers, are very elegant objects for electron microscope study. Two are visible in Fig. 2, Pl. I, and the uppermost of these is shown more highly magnified in Figs. 9 and 10, Pl. V. Close scrutiny of these last two figures will reveal the presence of numerous delicate double-headed hooks on long slender stalks scattered all over the outer surface of the skin. In shape they resemble boat-hooks or, to a botanist, the style and bifid stigma of the Compositae. Some very clear examples can be seen lying against the skin on the left of Fig. 10 or projecting from its edge on the right of this figure.

These hooks are completely invisible except at a rather high power of the electron microscope, but their effects can readily be seen even with the light microscope on living material. In a crowded suspension such as that described on p. 323 the jostling caused by the motile cells results in the immediate adherence of any cysts which are touched. A cyst may thus become attached to the back of the body of a motile cell and be carried about with it or a group of cysts may join together into a chain or floating raft. It may therefore be suspected with good reason that these very curious little structures may have a significant part to play in the natural dispersal of the species since they can presumably attach a cyst to any rough object or animal which may happen to touch it.

The front flagellum

Although in the arrangement of the plates it has seemed appropriate to put the micrographs of cyst skins in the proper time-sequence between those of the two motile phases, the detailed structure of the flagella in these is on the whole so similar that they can be dealt with together.

As already mentioned, the front flagellum is a *Flimmergeissel* in both phases. A complete first-stage flagellum is represented in Fig. 4, Pl. II, a complete second-stage flagellum (slightly decomposed as will be explained later) is shown at a higher magnification in Fig. 12, while portions of two rather well preserved first-stage flagella at approximately the same magnification are contained in Figs. 6 and 8. In all of these the hairs are very conspicuous. They extend almost to the tip of the flagellum (Figs. 4, 8, 12), and they are borne singly in two rows down the sides of the axis. Each hair ends in a very delicate hair-point and their total length, including the hair-point, is between 2 and 3 μ. They are therefore longer than in any other species examined by us except the Euglenoids (cf. Manton, 1951) and perhaps *Pylaiella*. The hair-points are visible in the original prints of all three high-power pictures (Figs. 6, 8, and 12), though it is possible that some of them may disappear in the process of half-tone reproduction. One of the clearest places is in the middle of Fig. 6, where the apparent thickness of other parts of the *Flimmer* hairs is enhanced by the presence of some gummy-looking additional material sticking to them. It is not known whether this material is an intrusion from without or a natural part of the organism which in other cases has washed away. The fact that it ends rather abruptly at a uniform distance from the axis might suggest the latter. The true hair-points, however, extend for a distance of about a centimetre, at the magnification of this picture, beyond the place of cessation of the gum.

A very conspicuous feature in Fig. 6 and elsewhere is the massive translucent sheath enveloping the bases of the hairs and through which the axis can be seen. A similar sheath can be seen in Fig. 4 and less clearly, owing to the reversal of printing, in Fig. 8. The sheath in Fig. 12, though still present, is partly decomposed. We were inclined at first to interpret this part of the flagellum as a raised skin, but we have found it so difficult to obtain any clear evidence that it is a tube and not a cylinder that the interpretation that it is a homogeneous secretion and not a skin seems the more probable. When dried down it contains so little solid matter that it forms a layer almost too thin to cast a shadow. After death of the cell it changes rather rapidly and it appears to liquefy, often running together into one or more drops along the length of the flagellum. A part of a detached flagellum which is behaving in this way is represented in Fig. 16, Pl. VIII. The curvature of the axis, induced presumably by surface tension exerted by the material of the sheath, is very conspicuous, but in other places where the sheath has stripped entirely away the *Flimmer* hairs are still attached to the now naked axis. This sheath will be discussed again in connexion with the hind flagellum.

The fibrillar nature of the axis has not yet been as fully demonstrated in this material as in most of the other organisms which we have handled previously. Nevertheless Fig. 12 gives some evidence about it which is indeed the reason for including this cell in the plates in spite of its partially decomposed state and of the risk that by using a direct, rather than a reversed, print some of the fine details of the *Flimmer* hairs may disappear. Decomposition in this specimen has affected not only the substance of the sheath throughout most of its length but the structure of the axis also. The component fibrils of this have begun to come apart laterally, especially near the tip. Here the surface of the sheath is still intact, but the axis within it has spread so as to occupy almost the whole interior. Five fibrils can be separately distinguished within an inch of the distal end and since with close packing of this kind it is unlikely that we are seeing more than half of the total number present we may safely infer that the total number, though still undetermined, cannot be far removed from that found in other cases, in all of which (cf. Manton, 1951) the number is of the order of ten and often exactly eleven.

The hind flagellum

There are fewer significant details to note about the hind flagellum than about the front one and in addition it is so long, especially on second-stage cells, that it cannot be fully recorded in one exposure except at a very low magnification. For this reason the only complete specimens are those of Figs. 1 and 3, though enlarged details of portions of these and of others will be found in Figs. 4, 5, 7, 12, 13, and 14.

In all of these a massive development of sheath, and an opaque central axis, are very evident. In addition there are points of interest about the tip, in which there is certainly a difference between first- and second-stage cells. In the first-stage specimen of Fig. 5 the thickness of sheath is gradually reduced towards the apex, but the axis remains of an almost constant width until it narrows abruptly about 1 μ from the end to form a short distal extremity. This narrow distal extremity is considerably longer in second-stage hind cilia, and it can indeed be seen readily with an ordinary microscope on a stained specimen (cf. Fig. 15) as has been described in the literature (cf. Couch). In the flagellum of Fig. 14 which belongs to the cell of Fig. 3 the narrow distal tip is about 3 μ long if the measurement is made from the external contour, but if the axis alone is measured the length is over 4 μ.

An instructive view of a comparable tip after removal of the sheath is contained in Fig. 17. In this the slender part of the central axis is freely exposed and it is again somewhat over 4 μ long. Some other details of this specimen will be discussed below.

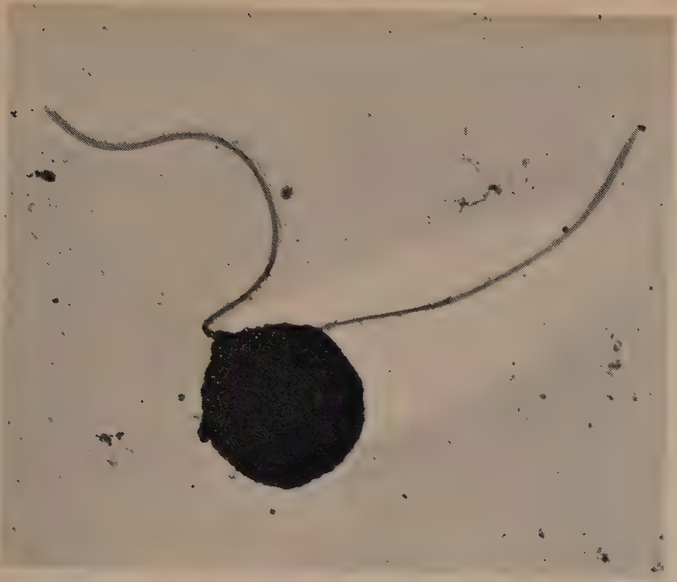
In addition to the details of the tip the only other facts which we have to record concern the sheath. This is very conspicuous in the upper parts of the hind flagellum of both phases as comparison of Figs. 7 and 13 will make clear. Here, however, we are in a difficulty which scarcely arose in connexion with the front flagellum, although it might perhaps have done so, namely that it is not always easy to determine, after an object has been dried, whether such flattening as it displays has been produced by the drying or whether it may also have been flattened in life. With regard to the front flagellum we have seen nothing tending to suggest that the flattening there was anything other than an effect of the drying, and, on the whole, the same may be said of the first-phase hind flagellum. With regard to the second-phase hind flagellum, however, the question of a possible flattening in life is at once raised by a specimen such as that of Fig. 13 (which is an enlarged portion of the cell of Fig. 3). Here the sheath is folded on itself at the end near the body and both sides are rolled in on themselves a little farther down where the flagellum passes out of the picture on the right. Both these appearances are more suggestive of a flat object which has become folded on drying rather than of a cylindrical object which has flattened.

Some further evidence of a totally different sort but tending to the same conclusion is contained in Fig. 17, though here the observations are so near to the limit of resolution of our machine as it is at present working that they will be difficult to reproduce convincingly in half-tone. This specimen is a hind flagellum still attached to a body but from which the sheath has stripped away from the apex, though it is still present as a spherical globule (outside the picture) nearer to the body. Upon the exposed axis, on the thicker part behind the distal extremity, an array of exceedingly fine hairs of uniform length has been exposed. They are about half a micron long and they are present all along the left-hand side of the part of the axis marked by arrows which point indeed to the tips of hairs as a precaution against their total disappearance in the making of the block. A more highly magnified reversed print of part of this picture is contained in Fig. 18 with the tips of hairs similarly indicated by arrows. In this particular part of the flagellum the hairs are visible on one side only. Higher up, however, they are visible in two ranks of equal length on the two sides of the axis and their length is not unlike the apparent width of the sheath in the dry condition. This observation clearly requires repetition and further work to confirm and extend it. It is, however, perhaps of sufficient interest to be mentioned in passing because it adds some additional evidence tending towards the suggestion that in the case of the hind flagellum of the second stage the flattening of the sheath may be present in life. The suggestion is in fact that it is a fin-like expansion along the two sides of the axis and that the fine hairs just discussed are perhaps an internal framework to this fin.

If this is true, and further confirmation is desirable, the very powerful swimming action of these zoospores, especially in the second stage, is to some extent explained and the differences in the swimming powers of second-stage cells compared with first must be interpreted as probably due more to the greater length and perhaps more elaborate structure of the hind flagellum rather than to any concomitant differences in the front flagellum or body.

DISCUSSION

We do not propose to discuss the observations in greater detail here because as they stand they are obviously incomplete in several respects, a deficiency which we hope shortly to make good. One further point may, however, perhaps be introduced concerning the comparison of electron microscope observations with those made with visual light. The existing works quoted in the first paragraph of the Introduction, which include previous observation on *S. ferax* itself (cf. Couch, 1941), together with our own measurements (cf. p. 327) on the rather unusual length of the *Flimmer* hairs on the front flagellum of this species made it seem profitable to attempt a direct comparison between visual and electron microscope views of the same material. Without elaborating this in detail at this stage, attention may perhaps be directed again to Fig. 15, Pl. VII. This is a visual light photograph taken with an oil-immersion objective in the usual way at the highest magnification which is significant with ordinary light. The specimen is stained with gentian violet following application of Kirkpatrick's bacterial flagellum stain (cf. Mackie and McCartney, 1942) used with double-strength mordant. For purposes of photography we have found this more satisfactory than Loeffler's bacterial stain as modified by Couch (1941), since it leaves less colour in the background. As a preliminary to staining by either method, the cells are killed and dried down on glass in a way somewhat resembling the treatment given before drying for examination with the electron microscope. The two types of material are therefore directly comparable and almost every detail of the one is interpretable in terms of the other. These details include the clear demonstration of two-sided *Flimmer*, although the 'hairs' are almost certainly not single but small tufts in the stained cell. The very wide sheath has an intense affinity for the stain, which on a first glance makes the width of the flagella seem exaggerated on the stained specimen. The dimensions are, however, quite comparable with the thickness of flagella shown by the electron microscope if the sheath is counted in. On a lighter print, or on a less intensely stained specimen, the central axis can be distinguished as a dark line within the paler sheath exactly as in the electron



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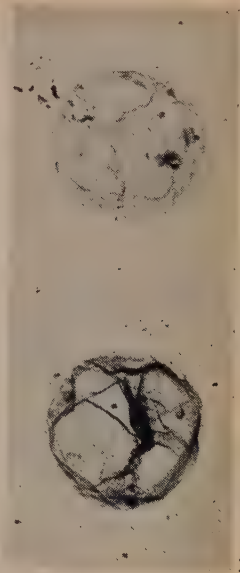

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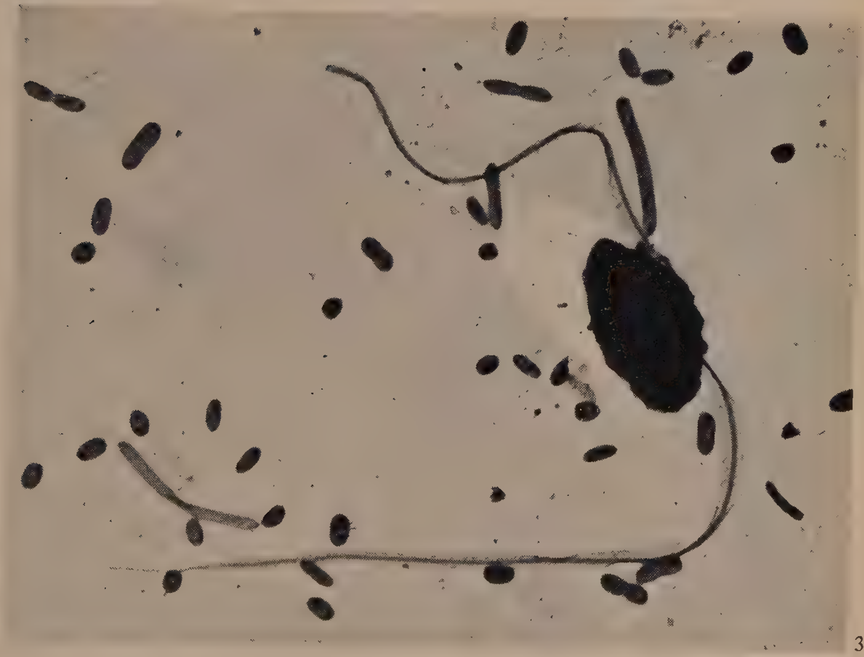

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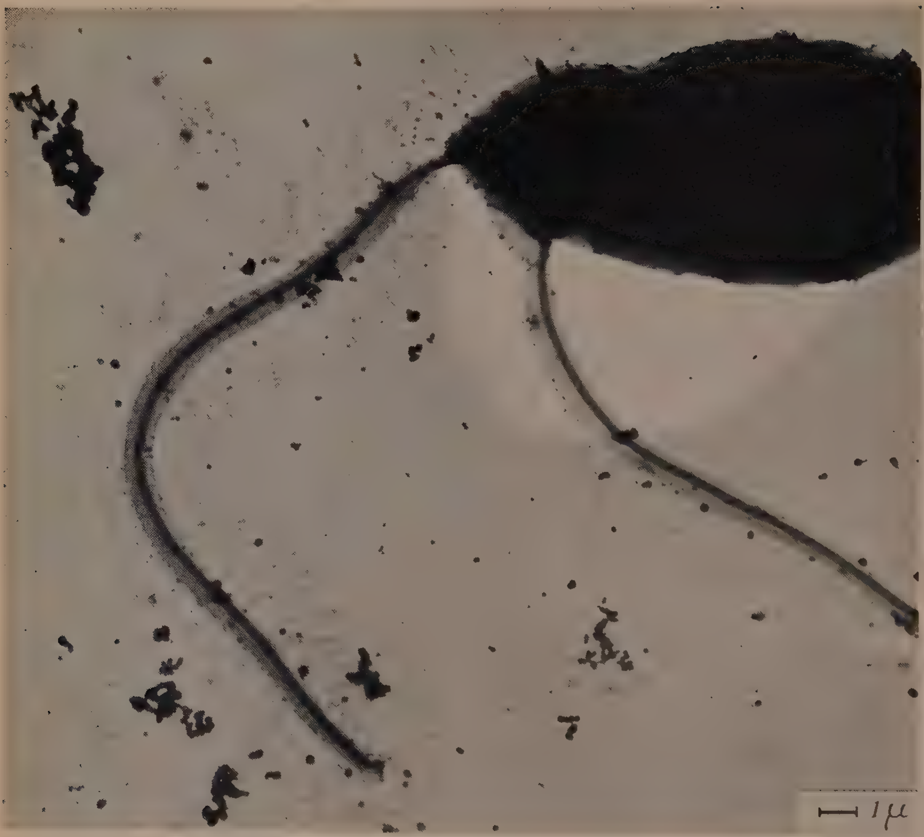
FIG. 1. *Saprolegnia ferax*, first-stage zoospore. Electron micrograph M44.15, 80kV., magnification × 3,000.

FIG. 2. Empty skins from encysted first-stage zoospores; further details of the upper skin are in Pl. V. Electron micrograph M45.32, 60kV., magnification × *c.* 3,000.

FIG. 3. Second-stage zoospore. Electron micrograph M45.17, 80kV., magnification × 3,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE I



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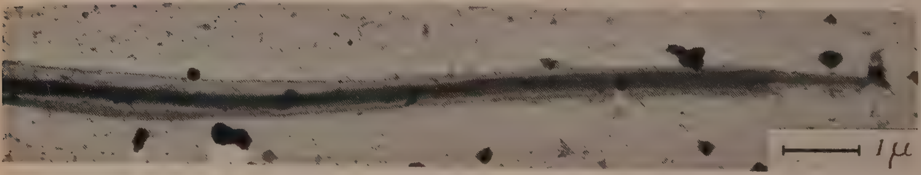

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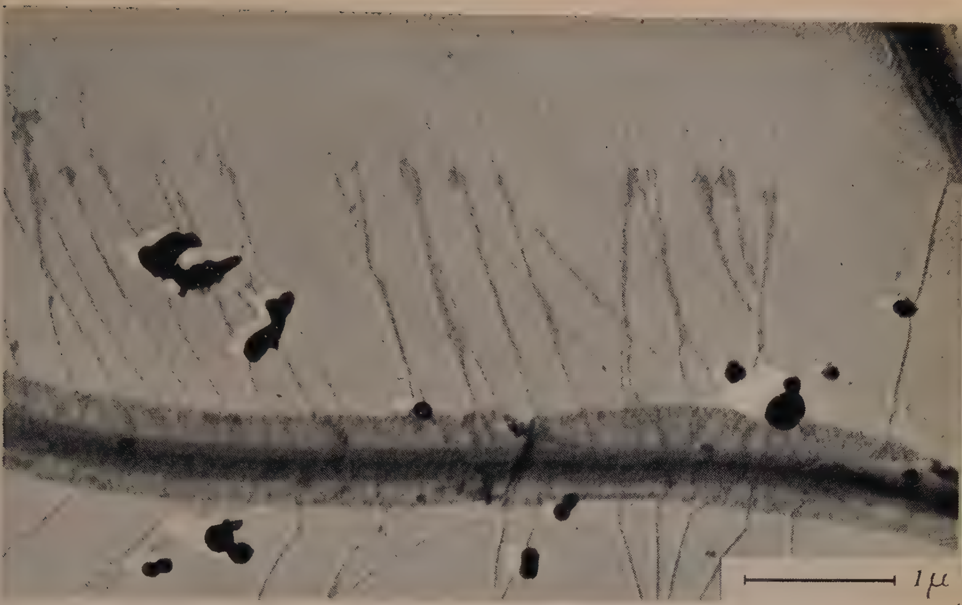
FIG. 4. *Saprolegnia ferax*. First-stage zoospore showing complete front flagellum. Electron micrograph M51.14, 60kV., magnification × 5,000.

FIG. 5. Distal end of the hind flagellum from a similar cell. Electron micrograph M51.10, 60V., magnification × 10,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE II



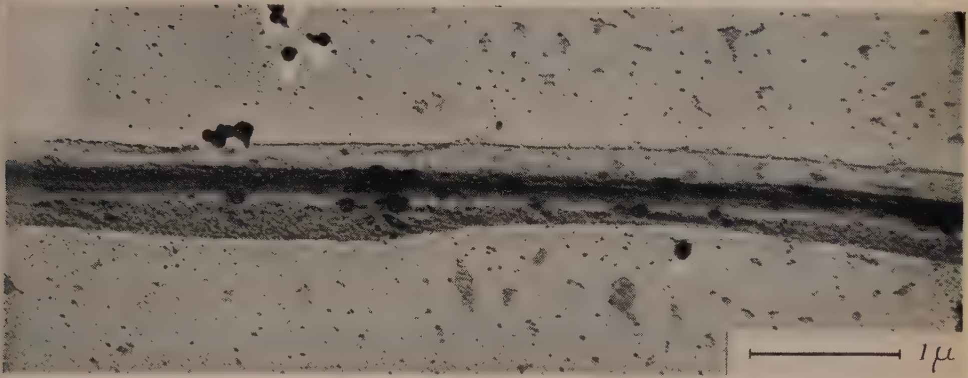


FIG. 6. Part of a first-stage front flagellum near the attachment to the body which is on the right. Electron micrograph M51.18, 60kV., magnification × 20,000.

FIG. 7. Part of a first-stage hind flagellum near the attachment to the body which is on the left. The tip of this flagellum is shown in Fig. 5. Electron micrograph M51.27, 60kV., magnification × 20,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE III

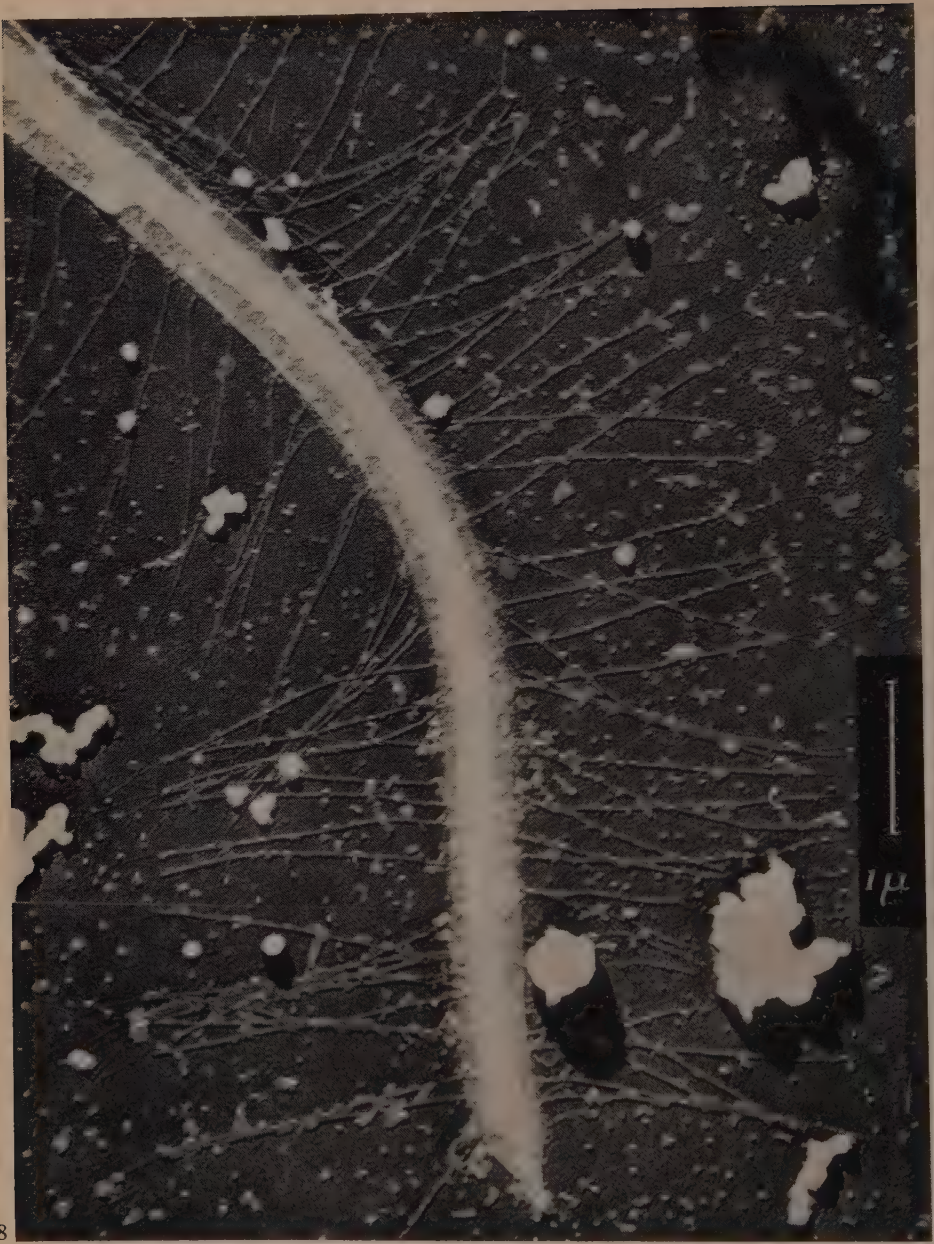


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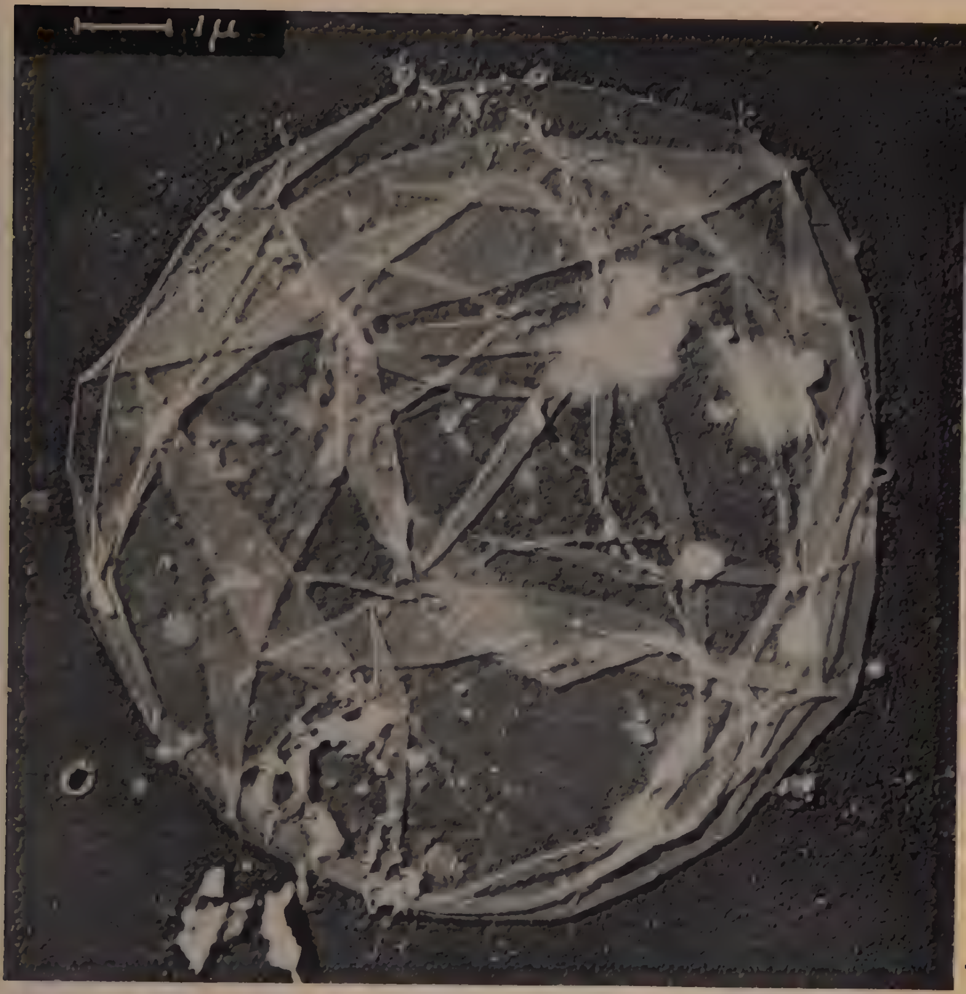
FIG. 8. *Saprolegnia ferax*, distal end of a first-stage front flagellum, reversed print. Electron micrographs M51.23 and 4, 60kV., magnification × 20,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE IV



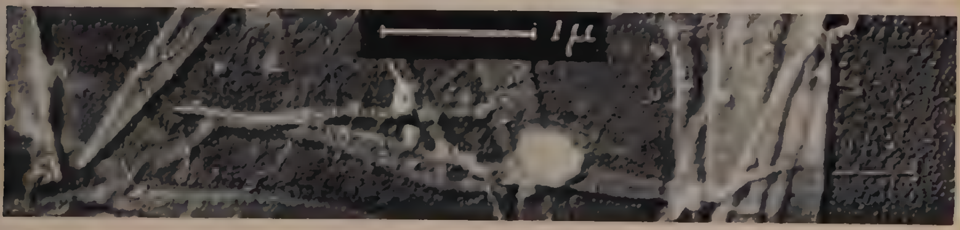


FIG. 9. An empty skin from the encysted stage which separates the two motile phases; more highly magnified view of the upper skin of Fig. 2. Note the slender hooks scattered over its outer surface. Electron micrograph M45.34, reversed print, 60kV., magnification × 12,500.

FIG. 10. More highly magnified portion of the above. Electron micrograph M45.31, reversed print, 80kV., magnification × 20,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE V

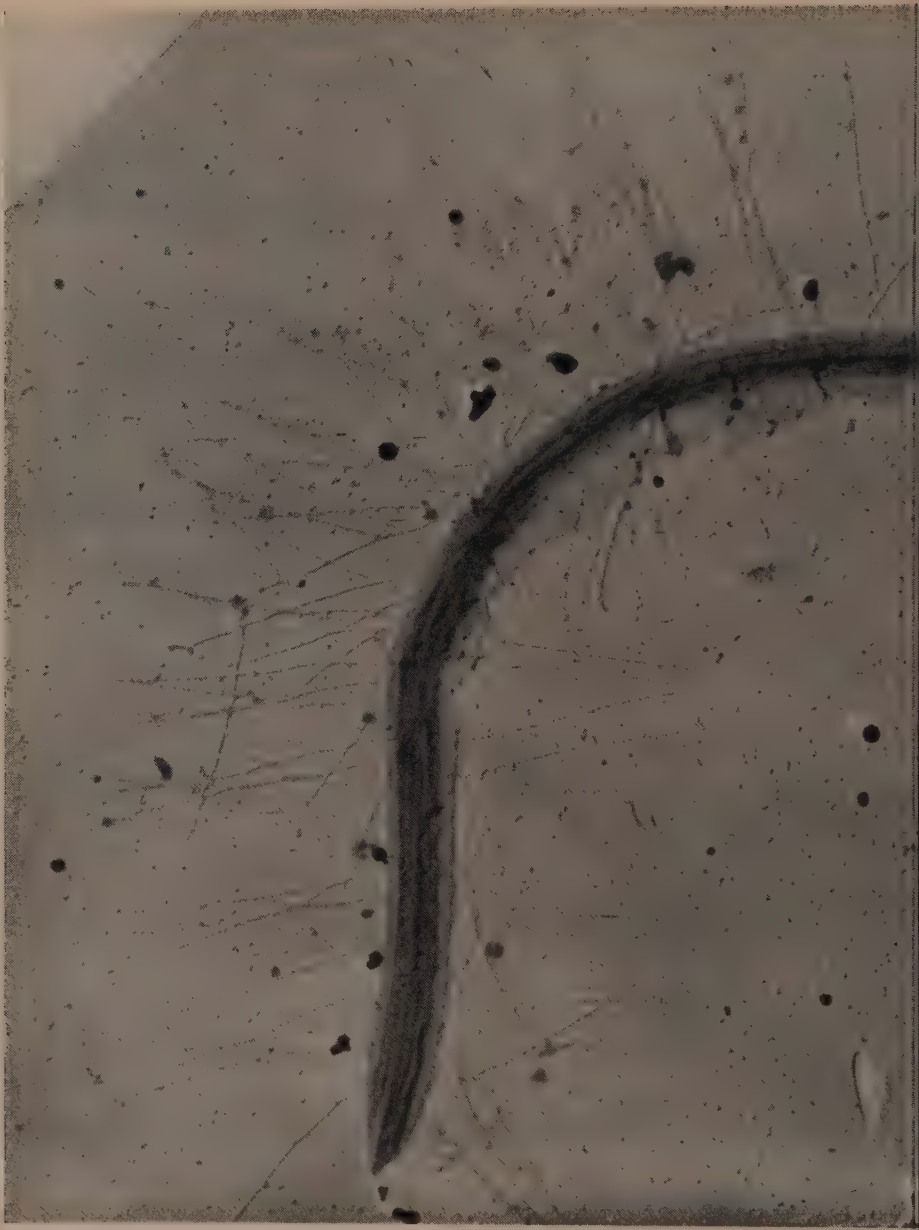


FIG. 11. *Saprolegnia ferax*, a second-st
Electron micrograph M54.6, 60kV., mag
FIG. 12. More enlarged view of the fro
and M55.8, both at 60kV., magnification

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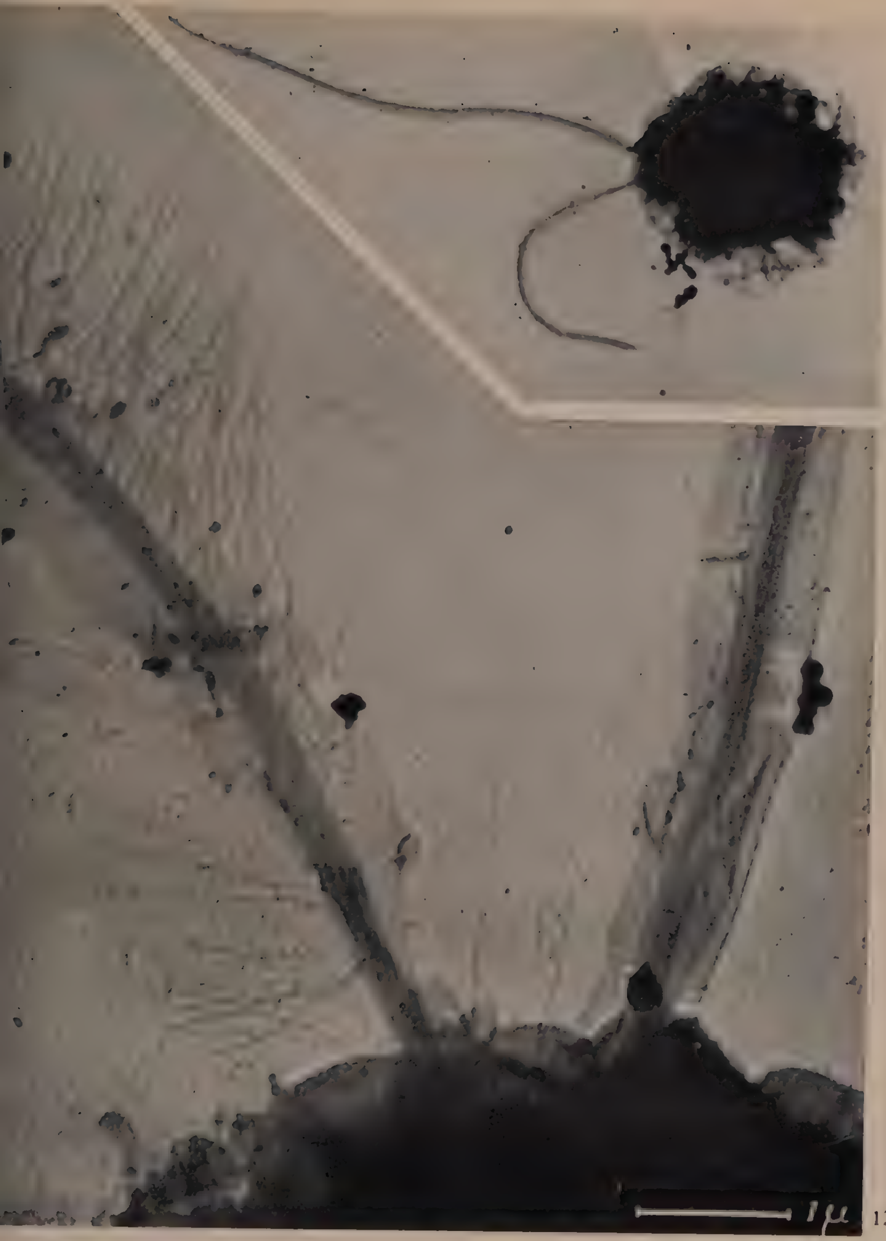

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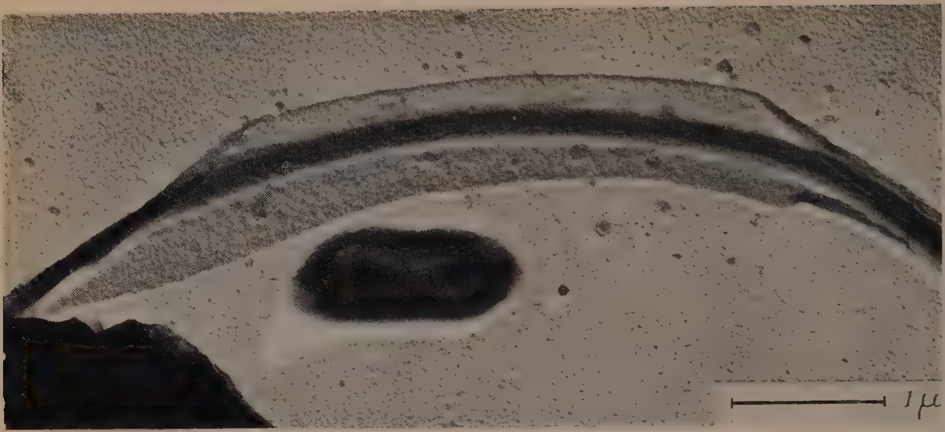
GREENWOOD



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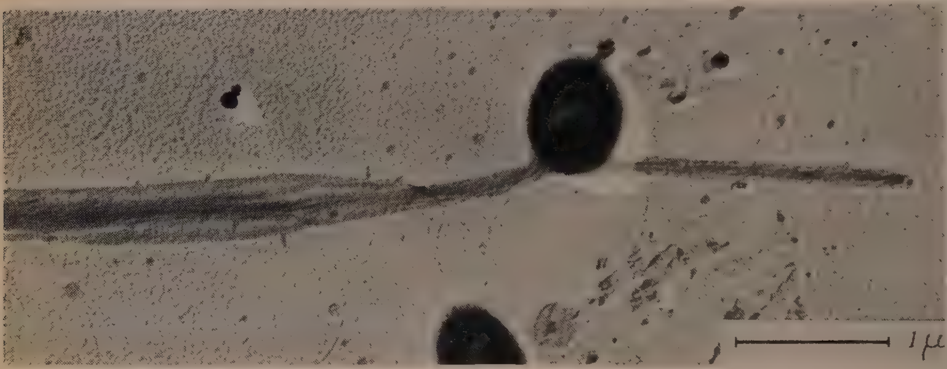

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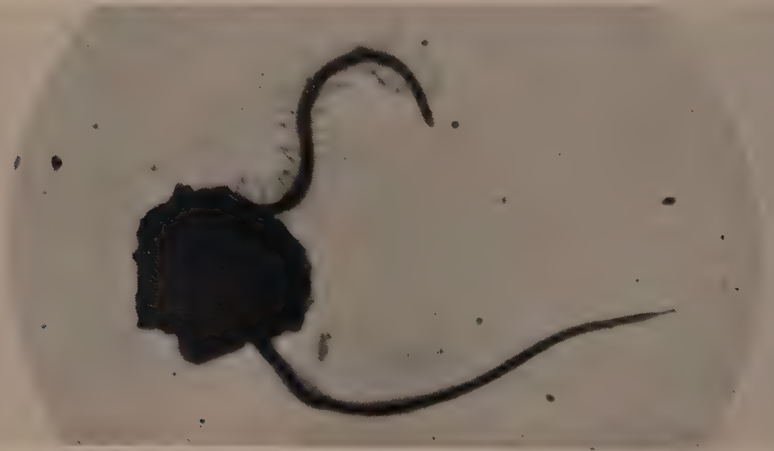

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FIG. 13. *Saprolegnia ferax*, part of hind flagellum of the second stage zoospore of Pl. I, Fig. 3. Electron micrograph M45.24, 80kV., magnification × 20,000.

FIG. 14. Tip of the hind flagellum of the cell of Pl. I, Fig. 3. Electron micrograph M45.25, 80kV., magnification × 20,000.

FIG. 15. Visual light photograph of a second-stage zoospore stained with gentian violet after a modified Kirkpatrick's bacterial flagellum stain. Magnification × 2,000.

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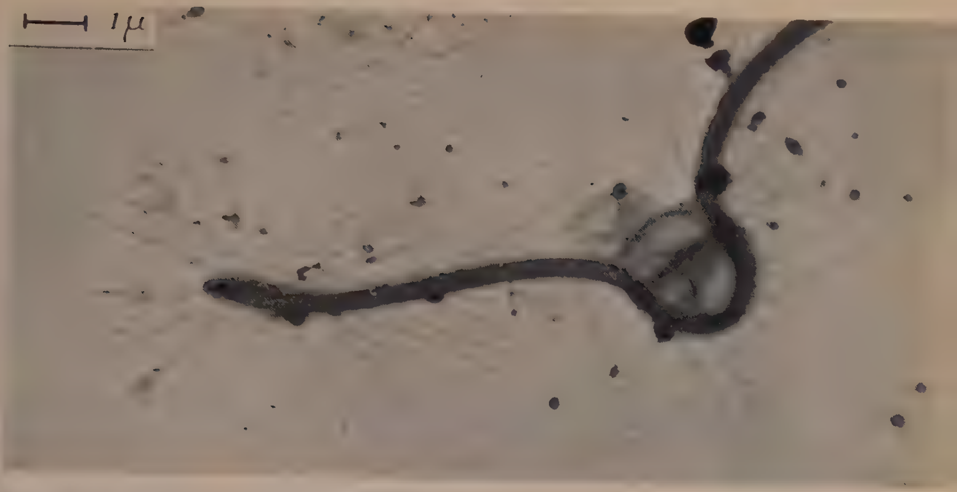
PLATE VII



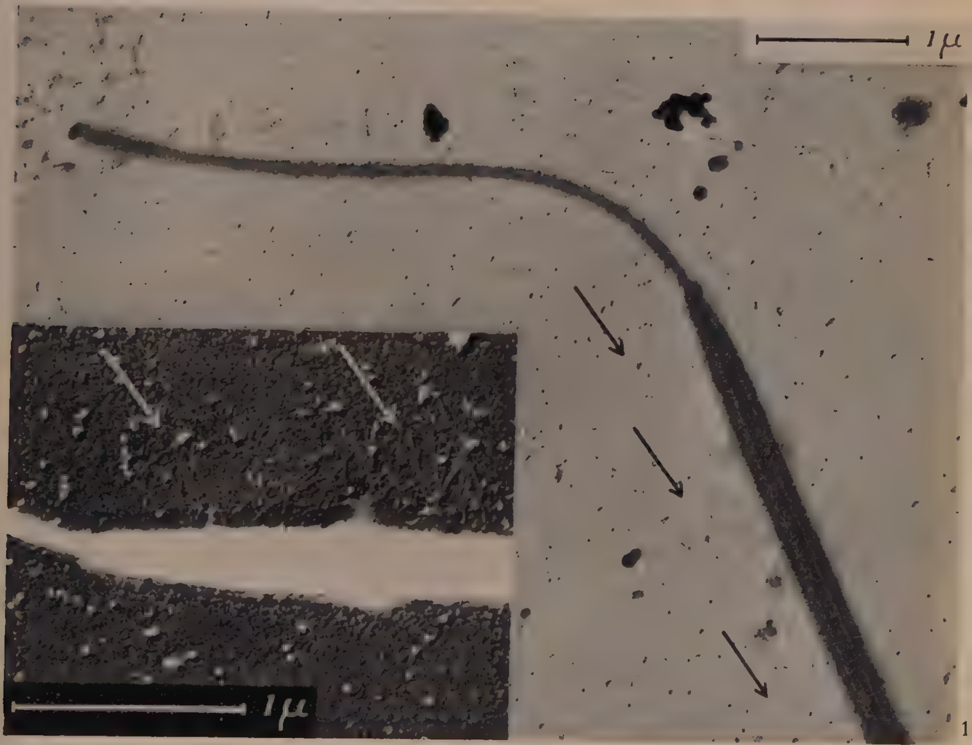


FIG. 16. Basal end of a detached front flagellum with the sheath partially stripped off and bending the flagellum axis where it remains. The Flimmer hairs are still attached directly to the axis. Electron micrograph M54.28, 60kV., magnification × 8,000.

FIG. 17. Tip of a second-stage hind flagellum exactly comparable to that of Fig. 14 except that the sheath has stripped away. On the left side of the thick part of the axis some very fine hairs are present, of a length extending to the tips of the arrows. Electron micrograph M55.18, 60kV., magnification × 20,000.

FIG. 18. Reversed print of a part of the preceding more highly magnified, magnification × 30,000.

I. MANTON, B. CLARKE, A. D. GREENWOOD

PLATE VIII

microscope pictures. Lastly the slender extremity of the hind flagellum has already been pointed out.

It is hoped to pursue these comparisons further on a later occasion. In the meantime it will perhaps reassure visual microscopists who may not possess an electron microscope at their elbow to see how well the visual light microscope and ordinary stains will stand up to comparison even as near to the limits of optical resolution as in this case.

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The Submicroscopic Structure of Pectin in Collenchyma Cell-walls

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WITH PLATES 1 AND 2

SUMMARY

Up to the present time pectin in cell-walls has been regarded as amorphous and optically isotropic. However, by means of polarization microscopy, X-ray diffraction, and electron microscopy, it is here demonstrated that the pectin in the fresh collenchyma cell-walls of *Petasites vulgaris* petioles consists of axially oriented submicroscopic fibrils containing axially oriented pectin crystallites and exhibiting negative intrinsic birefringence.

1. INTRODUCTION AND SURVEY OF LITERATURE

As is now generally accepted (Hottenroth, 1951) water-soluble pectin, or pectinic acid, consists mainly of straight chain-molecules of several hundreds of α–D–galacturonic acid radicals.

The optical properties of artificial pectinic acid fibres and films were first studied by van Iterson (1933). Fibres and sections of films were found to be positively birefringent[1] when mounted in water ($n = 1{\cdot}33$) or melted phenol ($n = 1{\cdot}54$), but faintly negative in air and Canada balsam. He also reported that X-ray diagrams, made by Burgers, disclosed the presence of oriented crystallites. This led to the postulation of rod-like pectinic acid crystallites, oriented parallel to the fibre axis or the film surface and possessing a faintly negative intrinsic double-refraction. This could be dominated by a positive form double-refraction on swelling of the inter-crystalline material, e.g. in water or melted phenol. Air and Canada balsam were supposed not to penetrate into the material. The optical properties were therefore similar to those already known for cherry gum.

This interpretation was confirmed in essence by Wührmann and Pilnik (1945), who found a negative minimum in the form double-refraction curve of synthetic fibres at $n = 1{\cdot}5$. They used non-swelling liquids such as xylene and dehydrated alcohols. The average refractive index of pectinic acid was found to be 1·50 and of de-esterified pectin (pectic acid) 1·53. The intrinsic double refraction of the latter material was stronger.

The crystallinity of pectinic acid, reported for the first time by van Iterson, has been confirmed and studied in more detail by other investigators, especially Wührmann and Pilnik (1945) and Palmer *et al.* (1947). The unit cell, proposed

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[1] The double refraction is called positive (resp. negative) if the longest axis of the index-ellipsoid is parallel (resp. perpendicular) to the axis of a fibre or a chain molecule or to the surface of a film.

Journ. of Experimental Botany, Vol. II, No. 6, pp. 332–343, September 1951.

by the latter investigators, involves a fibre identity period of 13 Å, comprising three galacturonic acid residues arranged on a threefold screw axis. The rodlet-shape of the crystallites as deduced from the form double-refraction was confirmed by the fact that the equatorial reflections were broader than the meridional ones.

The negative intrinsic birefringence of the pectinic-acid crystals is ascribed to the transversely oriented hydroxyl and carboxyl groups, whose effect on the polarizability apparently slightly dominates the effect of the pyranose chain, the latter effect being further reduced by the helical chain configuration. That this predominance of transverse polarizability is small is in accordance with the fact that the isomeric alginic acid is positively birefringent (Palmer *et al.*, 1947).

The positive flow birefringence of pectin solutions is explained by Pilnik (1946) as a suppression of the negative molecular double refraction by a positive form double-refraction due to formation of aggregates.

The above data apply to soluble pectinic acid. In the plant cell-wall, however, pectin occurs in an insoluble condition, often called protopectin. The essential chemical difference is unknown, but it is supposed that the methoxylated polygalacturonic acid chain molecules are not only longer, but are moreover intermolecularly (and perhaps also intramolecularly) connected through salt (Ca,Mg) bridges, ester-bridges, lactone-bridges, covalent hydrogen bonds, &c. Presumably the partial hydrolysis of these bonds liberates molecular fractions, which then dissolve, yielding pectin solutions.

Up till now the optical properties of cell-wall pectin have generally been considered as essentially different from those of artificial fibres and films, for both classical and modern microscopists agree that pure pectin layers in cell-walls are always optically isotropic. A known example is the pectin layer separating the cellulose layer and the cutinized layer in the outer cell-walls of the epidermis (Frey, 1926; Meyer, 1938). The cell-wall pectin, which is intermixed with cellulose and hemicellulose, is said to be isotropic also. Anderson (1927) stated that the pectin structures left after cellulose-extraction with cuprammonium hydroxide (Schweitzer's reagent) of collenchyma cells of tomato stems are completely isotropic. Similar isotropic pectin 'skeletons' have been obtained from many other objects, e.g. young cotton hairs, flax fibres, parenchyma cells, &c.

As far as we know, there is only one paper indicating the occurrence of double refraction in cell-wall pectin. Nakamura (1937) studied nuclear divisions in *Fritillaria* pollen mother-cells. After fixation in alcohol, embedding and cutting in paraffin, and mounting in cedar oil ($n = 1{\cdot}51$), he observed that newly formed middle lamellae were negatively birefringent. Cellulose in cell-walls never exhibits such a negative double refraction, but, as stated above, artificial pectin films do. Of course the observation of Nakamura is merely an indication of negative double-refraction of cell-wall pectin, for the transverse orientation of the long axis of the index ellipsoid might also be due to waxes or other lipids in the cell-wall. Many waxes are not dissolved by a mild treatment such as is used for obtaining the mounts. Lipids are known to occur in young cell-walls and, in the objects investigated so far, they are known to be oriented perpendicular to the cell-wall surface, e.g. in epidermal cell-walls (Meyer, 1938; Roelofsen, 1951; Sisson, 1938) and parenchyma cell-walls (Wührmann-Meyer, 1938).

As in the case of the optical properties, the crystallinity of cell-wall pectin also is considered to be different from that of artificial pectin fibres. Many investigators have obtained X-ray diagrams of cell-walls containing pectin in addition to cellulose, for instance fibres of flax, ramie, jute, oat coleoptiles, cambium cells, young cotton hairs. Disregarding the wax interferences in some of these, only cellulose interferences were visible and a diffuse blackening due to amorphous materials.

Preston and Duckworth (1946), who studied collenchyma bundles, do not mention the occurrence of non-cellulose interferences in their X-ray fibre diagrams either. This is the most striking example, since these bundles were reported to contain as much as 44 per cent. pectin.

Special attention may also be called to the work of Preston and Allsopp (1939), who irradiated several kinds of plant fibres before and after extraction of lignin, hemicelluloses, and pectin. They observed differences in sharpness of the cellulose diagrams only.

Admittedly there are records of unidentified X-ray interferences of cell-walls, for instance in conifer cambium (Preston and Wardrop, 1949), in jute (Sen and Woods, 1949), and in young cotton hairs (Sisson 1938), but as far as can be traced these correspond to spacings not found among those of artificial pectinic acid fibres.

However, the fact that no pectin interferences have been found is no proof that crystallized protopectin does not occur in cell-walls. Pectin ring diagrams show rather broad lines with about the same ring diameters as those of cellulose and are easily overshadowed by the latter. Pectin fibre diagrams differ markedly from cellulose fibre diagrams since two characteristic meridional interferences occur in the former. If, however, the degree of orientation of cellulose and pectin is moderate, these characteristic interferences merge into cellulose interferences.

X-ray diagrams of pectin-free cellulose cell-walls have been published in great numbers, but as yet no attempts to obtain diagrams of cellulose-free pectin cell-walls are known to us.

The generally accepted optical isotropism and amorphous state of cell-wall pectin caused us some surprise. If the pectin molecules were highly branched like glycogen and lignin, or consisted of a six-membered molecular ring as was supposed twenty years ago, then absence of double refraction and crystallinity would be more likely, although even completely amorphous lignin and silica 'skeletons' exhibit form double-refraction, because the dissolved cellulose leaves anisodiametric oriented pores (Freudenberg *et al.* 1929; Frey, 1926). The very long chain molecules, supposed to occur in cell-wall pectin, however, should have been oriented by cell-wall extension or during their deposition by oriented protoplasm as is the case with the cellulose molecules in the same cell-wall. Admittedly the admixture of pectin with much hemicellulose and cellulose might have interfered with pectin crystallization, but even amorphous pectin, if oriented, should exhibit both molecular double-refraction and form double-refraction. Since the former will presumably be negative and the latter positive, optical isotropism might occur in mounting media with a particular refractive index. This might account for the observation of optical isotropism in many cases, but in other media double-refraction is to be expected, although it might be faint.

Following this line of thought we decided to prepare pectin skeletons by dissolving the cellulose out of pectin-rich cell-walls and to study their submicroscopic structure, both optically, by X-ray diffraction and by the electron microscope. For this purpose we used the collenchyma bundles taken from the petioles of *Petasites vulgaris* L. Preston and Duckworth (1946) found that 44 per cent. of the dry bundles is pectin. The cellulose is reported to be evenly distributed throughout the cell-wall and perfectly oriented in an axial direction. In our opinion this suggests that here the pectin molecules will also run mainly axially.

2. PREPARATION OF PECTIN SKELETONS

Collenchyma bundles were isolated from fresh or alcohol-preserved petioles of *Petasites vulgaris*, preserved in 70 per cent. alcohol and later put in a bottle with cuprammonium solution (Schweitzer's reagent), prepared by adding concentrated ammonia to an excess of cupric hydroxide powder. Occasionally the bottle was shaken and every week the cuprammonium was renewed. To check the cellulose-extraction, samples from this stock were washed in concentrated ammonia, cold 1 per cent. HCl and 70 per cent. alcohol, mounted in this liquid and examined between crossed nicols. The initially bright, positive double-refraction rapidly diminished and disappeared from the outer parts of the bundle, but it took 1–2 months before the extraction process was complete in the central part. Then practically the whole bundle exhibited a very small positive double-refraction, clearly visible only by applying the sensitive method of Laves and Ernst (1945); ($n\gamma$ of the red *I* compensator orientated not diagonally but at a small angle with the polarizer vibration plane). The bundles shrank considerably during the extraction. When completely extracted, they were stored in absolute alcohol dehydrated by boiling with CaO.

Such extracted collenchyma bundles did not contain any cellulose except in some adhering lignified wood-vessels and a minute quantity of precipitated cellulose granules occluded in cells in the centre of the bundle: the cellulose sphaero-crystals described by Gilson (1893). Absence of cellulose was shown by dissolving the tissue in 5 per cent. H_2O_2 at 50° C. (Kisser's maceration method), or in 2 per cent. NaOH at 100° C. or in hot 1 per cent. HCl. If the residue was dried on a slide and mounted in chlorzinc iodine, no cellulose cell-walls could be found, but only 'dirt' in addition to some violet-coloured Gilson-crystals and occasionally some lignified spiral thickenings.

These crystals did not at all affect the optical properties of the pectin skeletons since their amount was small and their double-refraction practically nil. Moreover, if double-refraction occurred in the pectin skeletons, this was visibly located in the cell-wall, which did not contain cellulose crystals.

A sufficient amount of such crystals might interfere with X-ray investigation of the extracted bundles, but in our objects there were too few of them (see further, section 4).

3. OPTICAL PROPERTIES OF PECTIN SKELETONS AND CONCLUSIONS TO BE DEDUCED THEREFROM

A cellulose-free bundle from the stock in absolute alcohol was mounted in this liquid and a rough sketch was made of its shape. Then the retardation was measured in one or more suitable locations, using a Leitz elliptic compensator provided with a 0·1λ platelet. There upon the bundle was carefully transferred by means of a hair brush into a glass tube containing one of the other liquids mentioned in Table I. After a while this liquid was renewed and the tube was kept closed for at least 2 days before the retardation was again measured on the same spots. This procedure was repeated with other mounting liquids till the bundle had been damaged too much and had to be discarded. Several bundles were examined in this way.

Since spots in different bundles and even different spots in the same bundle vary in thickness, only retardation figures of the same spot in different mounting liquids are strictly comparable. However, the pectin skeletons were very delicate and they were usually damaged before the whole series of mounting liquids was covered. In order to compare different series and to obtain averages, the retardation figures were converted to a standard thickness. Since the absolute thickness could not be measured, we chose the thickness giving a retardation in abs. alcohol of 12 mμ, being the average retardation actually measured in this liquid. Only figures obtained from the same lot of bundles were averaged, because in different lots a varying degree of crystallinity and of porosity seemed to occur. The results are given in Table I.

Most liquids penetrated readily into the pore-structure of the pectin skeletons, as could be judged from the fact that often the retardation practically attained its final amount after an immersion of only a few minutes. Some very lipophilic apolar liquids such as CS_2 and especially 1-bromo-naphthalene, however, penetrated badly. They often penetrated only into the peripheral parts of the bundles, as was apparent from the fact that the expected change in the character of the double-refraction only occurred in these spots. With 1-bromo-naphthalene no improvement resulted from heating for some hours at 100° C. or for some days at 50° C. Apparently diffusion velocity is not the limiting factor.

The difficulty of penetration of apolar liquids into cellulose and starch has been emphasized—possibly too much—by Frey-Wyssling and Speich (1942).

TABLE I

Retardation of cell-wall pectin skeletons in different mounting liquids. Retardation in mμ, converted to standard thickness giving 12mμ in alcohol. The figures demonstrate the occurrence of form double-refraction and intrinsic double-refraction; + (—) means nγ//(⊥) bundle.

Mounting liquid	*nD*	Retardation (mμ)	Mounting liquid	*nD*	Retardation (mμ)
Methanol	1·32	+16·5	Bromobenzene	1·560	−4
Water	1·33	+9	Aniline	1·584	−5
Ether	1·354	+12	Cinnamomum oil	1·589	+4·5
Ethanol	1·363	+12 (+5)	Styrax	1·59	—
Amyl alcohol	1·408	+6·5 (−2)	Bromoform	1·598	−5
Glycol	1·428	+3 (−4)	Quinoline	1·620	−4
Glycerol	1·473	−1 (−7·5)	Carbonbisulfide	1·623	+3·5
Benzene	1·500	−6·5	1-bromonaphthalene	1·658	+6
Methylbenzoate	1·512	−6	Thoulet solution	1·71	destruction
Canada balsam	1·535	—			
Clove oil	1·537	−6			
Benzyl alcohol	1·538	−3·5			

As is generally experienced in microtechnique, such apolar liquids readily penetrate hydrophilic tissues if a polar liquid, e.g. absolute alcohol, is used to remove water and to 'lead the way' for the apolar liquid. This phenomenon has been extensively studied with cellulose by Stamm and Tarkow (1950), who supposed that a unimolecular layer of such a polar precursor was retained on the walls of the pore spaces.

In view of these considerations we always started our imbibition series with absolute alcohol, but apparently this did not guarantee complete penetration of all apolar liquids.

There are some notable deviations among the figures given in Table I. These will be considered later, but without any doubt the general trend leads to two conclusions:

(*a*) there is a negative intrinsic double-refraction of the pectin;
(*b*) there is a Wiener effect due to a positive form double-refraction, disclosing the presence of axially oriented rodlets and/or pores.

The intrinsic double-refraction of the pectinic material must be the result of oriented pectin molecules, possibly partly aggregated into crystalline rodlets (crystallinity actually occurs, see section 4). The molecules must be negatively birefringent and oriented axially, which may be deduced from the following observation. If a pectin skeleton or one collenchyma cell, mounted in benzene for instance, is observed at high magnification, the cell-walls are negatively birefringent both in tangential and in surface view. If the orientation were transverse, a surface view and a tangential view of the cell-wall should exhibit double-refraction of opposite character, both with positively and with negatively birefringent molecules. Moreover, transverse orientation of pectin would be difficultly reconcilable with axially elongated pore spaces, which actually occur, as will become apparent. Thus the molecules of our pectin skeletons are negatively doubly refractive like the pectinic acid molecules of the artificial fibres obtained by previous investigators.

Since the protopectin will have been rapidly de-methoxylated in the concentrated ammonia used and was subsequently washed with acid, the pectin skeletons will be composed of pectic acid and probably some calcium pectate in addition to a small quantity of cuprammonium-insoluble hemicelluloses.

As regards the origin of the form double-refraction, we may draw the following conclusions. Both polar and apolar imbibition liquids apparently affect the form double-refraction. Since the apolar liquids presumably will not penetrate either amorphous or crystalline pectin, this discloses the presence of axially elongated pores (actually visible on the electron micrographs to be discussed in section 5). In the native cell-wall, obviously much pore space will have been filled with cellulose fibrils or at least cellulose crystallites.

The polar imbibition liquids, e.g. water, alcohols, glycol, and glycerol, will also penetrate the pores and affect the form double-refraction on the boundary of the pore spaces and the pectin. In addition, these liquids might penetrate amorphous pectin and possibly even the crystalline pectin (Palmer *et al.*, 1947). Unequal penetration of amorphous and crystalline pectin obviously will produce form double-refraction or affect already existing form double-refraction, on the boundary between these pectin fractions. This was already reported by van Iterson (1933) from studies of artificial pectinic acid fibres. These considerations suffice to show that the effect of polar imbibition liquids may be complicated.

Now the deviations in the figures in Table I will be discussed briefly. The figures between brackets were obtained with a different sample of extracted collenchyma bundles. They demonstrate that the magnitude of the intrinsic double-refraction and of the form double-refraction may vary considerably. This fact is to be expected and essentially the difference observed is irrelevant, but it carries a warning that the measurements obtained with different lots should not be averaged.

Furthermore, one gets the impression that the behaviour in certain mounting liquids is abnormal. In water the double-refraction is too low; swelling effects and penetration into crystallites may be responsible for this. As van Iterson observed, artificial fibres mounted in water are even negatively birefringent.

There is also a divergence in the behaviour in cinnamomum oil, CS_2, and 1-bromonaphthalene on the one hand and aniline and Styrax (*Liquidambar* resin), bromoform and chinoline on the other. As a result the real course of the form double-refraction curve in the region between $n = 1{\cdot}54$ and $1{\cdot}62$ is uncertain. Insufficient penetration of pores may play a part, as well as oriented adsorption or chemical reaction as might occur between carboxyl groups and basic substances (aniline, chinoline). Abnormal phenomena due to adsorption have been observed in many cases, e.g. in chitin, gelatin, and collagen impregnated with cinnamomum oil, phenol, resorcin, and certain tannins; in cellulose with organic nitrogen bases and in nitrocellulose with aromatic liquids.

4. THE EVIDENCE OF CRYSTALLINITY[1] IN THE COLLENCHYMA CELL-WALL PECTIN

a. Pectin skeletons

Pectin skeletons similar to those examined optically (see section 2) were immersed in 40 per cent. alcohol, transferred to a slide, and piled up to form a packet of 10–15 parallel bundles. The packet was either dried on the slide or it was dried while fixed in a fibre-stretching apparatus after it had been stretched as far as seemed possible without rupture. This was 20 per cent. at most.

Thus strips of 1–2 mm. broad and 0·1–0·25 mm. thick were obtained. X-ray diagrams were made by irradiation perpendicular to the flat side (Ni-filtered CuKα radiation, pinhole 0·5×40 mm., specimen film distance 4 cm.).

X-ray powder diagrams of pure pectinic or pectic acid show three characteristic rings, viz. a rather diffuse inner ring corresponding to a spacing varying between 6·5 and 7·1 Å; a moderately intense, rather sharp, intermediate ring corresponding to 4·20–4·18 Å; and a weak outer one corresponding to ±3·50 Å. They are shown in Pl. I, Fig. 1 A, and indicated by PE, PM_1, and PM_2 respectively, because in fibre diagrams of pectinic and pectic acid fibres the first one shows a maximum on the equator and the other ones on the meridian. The inner interference is assumed to be connected with the interchain spacing; its position varies with the methoxyl content and may also be influenced by other factors, e.g. humidification (Palmer *et al.*, 1947).

The unstretched packets of pectin skeletons produce ring interferences with very little evidence of orientation (quadrant *B* of Pl. I, Fig. 1). The ring diameters correspond to those of Pl. I, Fig. 1 A, as is especially clear for PM_1. PE is overshadowed by diffuse blackening from impurities and PM_2 is very weak and not visible on the reproduction.

The general agreement between the diagrams is no proof, however, that the interferences of Pl. I, Fig. 1 B, are due to pectin. In section 3 the presence of a small number of cellulose sphaero-crystals in the pectin skeletons was reported. As is illustrated by Pl. I, Fig. 1 C, similar crystals[2] prepared from beetroot tissue produce cellulose II interferences. Since apparently the diameters of the main ring interferences of cellulose II are but slightly different from those of pectin, further evidence is required that the interferences of Pl. I, Fig. 1 B, cannot be due to the cellulose crystals. This might have been expected on account of the fact that the number of crystals in the pectin is very small. It is definitely proved, however, by the diagram obtained from a stretched packet of skeletons, since this reveals preferred orientation in the specimen; note the clear meridional maximum of PM_1 in Pl. I, Fig. 2. It is clear that sphaero-crystals with their radially oriented crystallites can only produce homogeneously blackened rings. Furthermore, even if oriented cellulose crystals would occur, the maxima of its two main interferences should be found on one line, which is not the case in Pl. I, Fig. 2. Hence we may conclude that the pectin skeletons contain crystalline pectin.

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[1] In this paper 'crystalline' is understood as 'producing X-ray interferences'.

[2] These were obtained as follows. Sections of 30 μ thickness from beetroot tissue were kept in cuprammonium until they were practically isotropic. They were washed in conc. ammonia and water. Then the cell-wall pectin was dissolved in 1 per cent. HCl at 100° C. for 15 minutes. After centrifuging the sediment was heated in 3 per cent. NaOH at 100° C. for 2 hours and washed in water, alcohol, and ether. Finally the remnants of lignified wood vessels were removed under a dissecting microscope.

If we have a further look at Pl. I, Fig. 2, it appears that the innermost reflection is more clearly shown than in Pl. I, Fig. 1A. It is slightly accentuated in the equator. On the original photograph one could moreover observe a weak outer reflection which was slightly intensified in the meridian. The intermediate and outer reflections correspond to spacings of 4·18 and 3·09 Å respectively. All this is in accordance with the data of Palmer for oriented fibres of pectinic and pectic acid. The side spacing derived from *PE*, however, is larger than is usual, i.e. ±8 Å instead of 6·5–7 Å. Hence the chains are wider apart than in pure preparations.

Apparently the degree of orientation of the pectinic acid crystallites in stretched pectin skeletons is low, probably as a result of disorientation accompanying the shrinkage during cellulose extraction. This shrinkage was a multiple of the 20 per cent. stretch that could be reached with the packets of pectin skeletons.

b. The pectin in the native cell-wall

The presence of axially oriented pectic acid crystallites in the dried pectin skeletons is no proof of the presence of crystalline pectin in the native cell-wall.

Firstly, crystallization might have taken place after extraction of admixed cellulose because the removal of cellulose molecules might allow the protopectin molecules to aggregate. A similar phenomenon has been reported to occur in other cases, for instance with cellulose in jute fibres on extraction of hemicelluloses (Sen and Woods, 1949) and in cambium cell-walls on pectin extraction (Wardrop, 1949).

Secondly, amorphous cell-wall pectin might have crystallized on drying. Crystallization as a result of dehydration has been reported to occur in other instances, e.g. with dextran (Jeanes *et al.*, 1948), and even with native cellulose (Berkeley and Kerr, 1946).

However, examination of Pl. I, Figs. 3, 4, and 5, will lead to the conclusion that pectin is actually crystalline in fresh native collenchyma bundles.

The first of these diagrams was obtained from a packet of fresh collenchyma bundles kept wet during preparation and irradiated in an atmosphere of saturated water vapour. No stretch was applied.

The second diagram was obtained from the same packet immediately afterwards, but now allowed to dry out during the irradiation. After the exposure (1 hour) it was still slightly humid.

The third diagram was produced by a pectin-free bundle, obtained by treating a fresh bundle successively with water at 50° C. for 24 hours, boiling alcohol for ½ hour, boiling chloroform for ½ hour., 2 per cent. NaOH at 100° C. for 3 hours, 1 per cent. HCl at 100° C. for ½ hour, 5 per cent. H_2O_2 at 50° C. for 24 hours. This 'cellulose skeleton' was slightly stretched, dried, and irradiated.

The packets of bundles were about 1 mm. thick in the wet state and the exposure time was 70 minutes for Ni-filtered CuKα radiation at 110 mA/35kV, with a pinhole 0·5 × 40 mm. and a specimen-film distance of 4 cm.

Obviously Pl. I, Fig. 5, represents a fibre diagram of cellulose I. Special attention is called to the rather clear meridional region between the (021) interferences.

Pl. I, Figs. 3 and 4, exhibit the same diagram on which the pectin diagram of Pl. I, Fig. 2, is superposed in addition to a diffuse water halo. This is evident from the meridional maximum between the cellulose (021) interferences. If the pectin were amorphous, then in Pl. I, Figs. 3 and 4, merely a diffuse blackening should be superposed on the cellulose diagram. The meridional pectin reflection may be regarded therefore as convincing evidence of the crystallinity of at least part of the cell-wall pectin in the native collenchyma bundles. Hence the crystallinity observed in the pectin skeletons is not a result of desiccation or cellulose-extraction.

Furthermore, we may conclude from Pl. I, Figs. 3 and 4, that in the native collenchyma fibre the pectin crystallites are highly oriented; otherwise no such a clear meridional maximum would appear.

Admittedly the occurrence of crystalline cell-wall pectin in the object examined is no proof that cell-wall pectin is crystalline in other objects. In particular in cell-walls with a low pectin content and with isotropic structure pectin might in fact be completely amorphous, as was supposed up to the present. However, the comparative difficulty of detecting the pectin interferences in X-ray diagrams of cell-walls might also explain the general acceptance of the amorphous condition of cell-wall pectin.

Although crystalline cellulose was reported to occur in fresh cell-walls (Heyn, 1934; Hess, 1936), this was later denied (Berkeley and Kerr, 1946), but recently (Preston *et al.*, 1948) it was demonstrated again in a convincing manner. However, as far as we know, there have never been published diagrams of fresh native cell-walls which demonstrate as clearly as Pl. I, Fig. 3, that cellulose may actually be highly crystalline in fresh native cell-walls.

Finally, we wish to note that on the original of Pl. I, Fig. 4, some unidentified reflections are visible which become stronger when the specimen is completely dried and disappear after extraction with water at 50–65° C. for some days. Their origin is being investigated.

5. ELECTRON MICROGRAPHS OF PECTIN SKELETONS

Since apparently the crystallites of cell-wall pectin are rod-like and are axially oriented, we wondered whether cell-wall pectin would prove to be fibrillar or not. On our request Dr. A. L. Houwink of the Institute for

Electron Microscopy at Delft has prepared some electron micrographs. Pectin skeletons from collenchyma bundles were teased apart, dried on to a Parlodion covered mounting-grid, shadowed with chromium, and examined.

Pl. II, Figs. 6 and 7, are typical examples of the pictures obtained. There might occur some hemicellulose in these pectin skeletons, but the abundance of fibrils leaves no doubt that it is the cell-wall pectin which is fibrillar. The demonstration of a fibrillar structure in cellulose-free dry pectin skeletons by itself does not furnish evidence of the presence of the fibrils in the *fresh* cell-wall. Pectin molecules and crystallites might aggregate to fibril-like bundles after extraction of cellulose or as a result of drying. However, on account of the rather uniform fibril thickness and the fact that, according to the X-ray diagrams, the degree of crystallinity has not clearly increased in the dry skeletons as compared with the native bundles, this possibility is considered extremely improbable. In fact, as improbable as the non-existence of cellulose-fibrils in fresh cell-walls.

In surface view the cell-walls in the pectin skeletons show an arrangement of the fibrils that appears to be practically at random (as reported in section 3, optical examination has shown that in reality a small predominance of axial orientation occurs). On tangential view the fibrils of course are parallel to the direction of the cell-wall. Since the collenchyma cells are long and thin, axial fibril orientation will predominate in the pectin skeletons of collenchyma bundles, but obviously the degree of orientation is not high, for their double-refraction is small and, when unstretched, their X-ray diagrams show very little orientation.

On the other hand, a high degree of axial orientation must occur in the native cell-wall, otherwise the meridional maxima of the PM_1 reflection visible in Pl. I, Figs. 3 and 4, should be less distinct. This difference in degree of orientation of the pectin fibrils between the skeletons and the native cell-walls must be a result of the considerable shrinkage during cellulose extraction and drying.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. A. L. Houwink and the Institute for Electron Microscopy at Delft for preparing the electron micrographs and to Professor L. J. Audus of London for his correction of the English text.

Both the electron microscope and the X-ray work have been supported financially by the Netherlands Organization for Pure Research (Z.W.O.).

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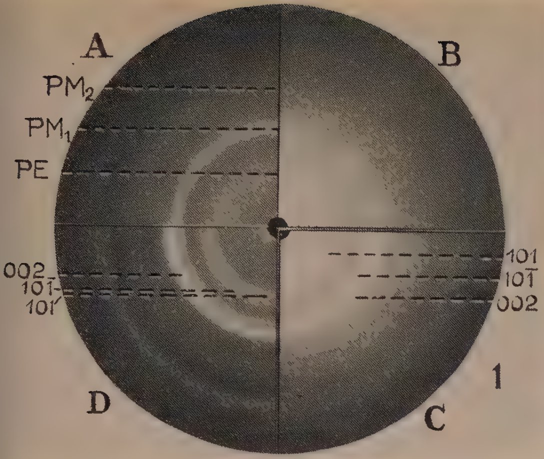

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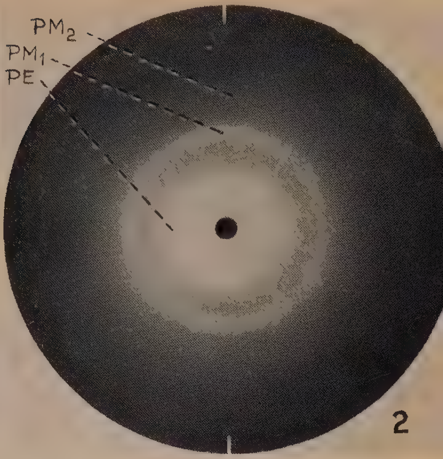

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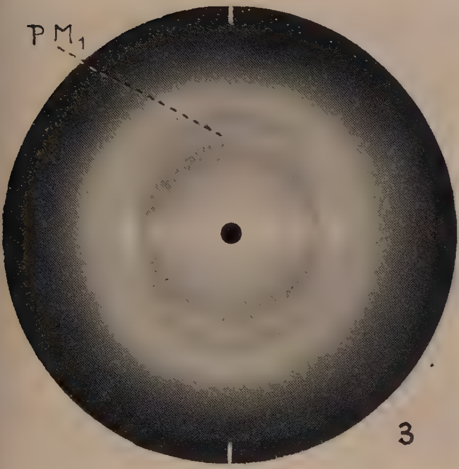

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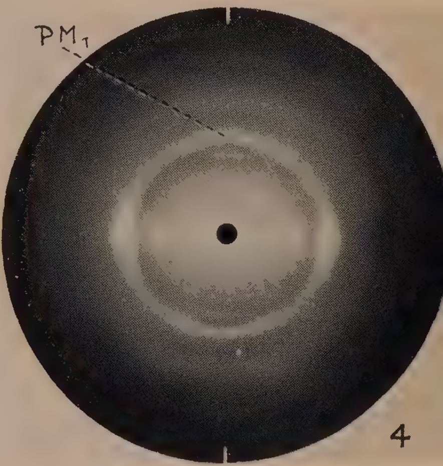

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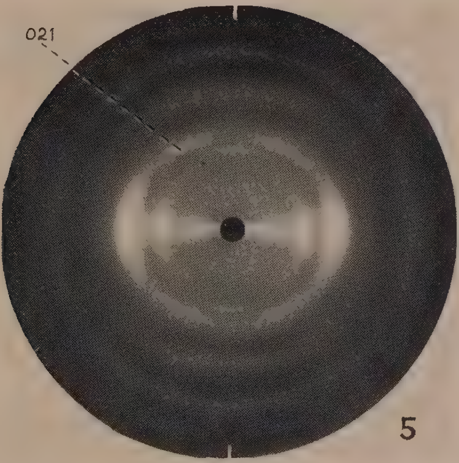

5

P. A. ROELOFSEN AND D. R. KREGER—PLATE I

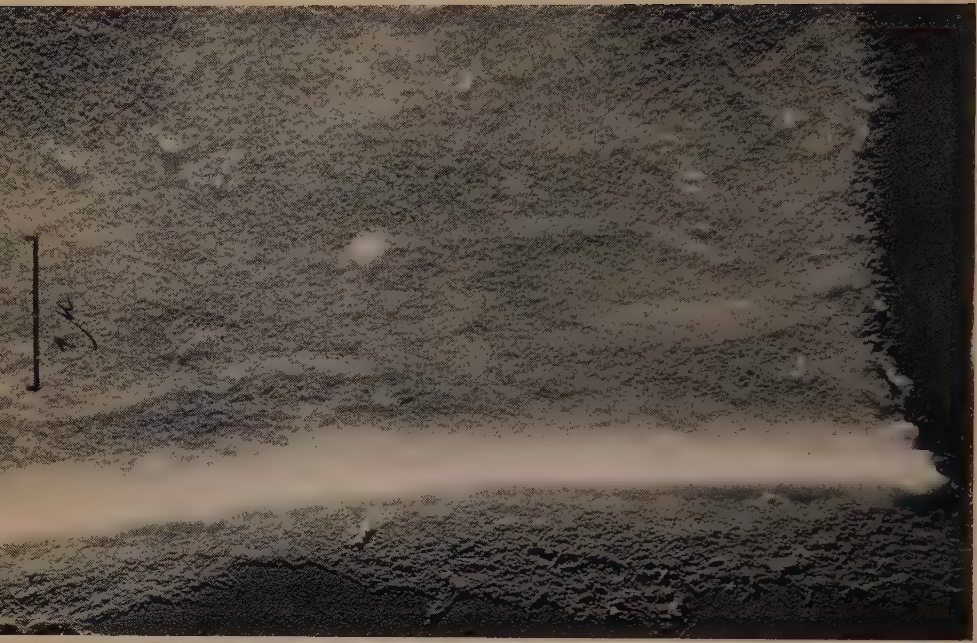

FIG. 7

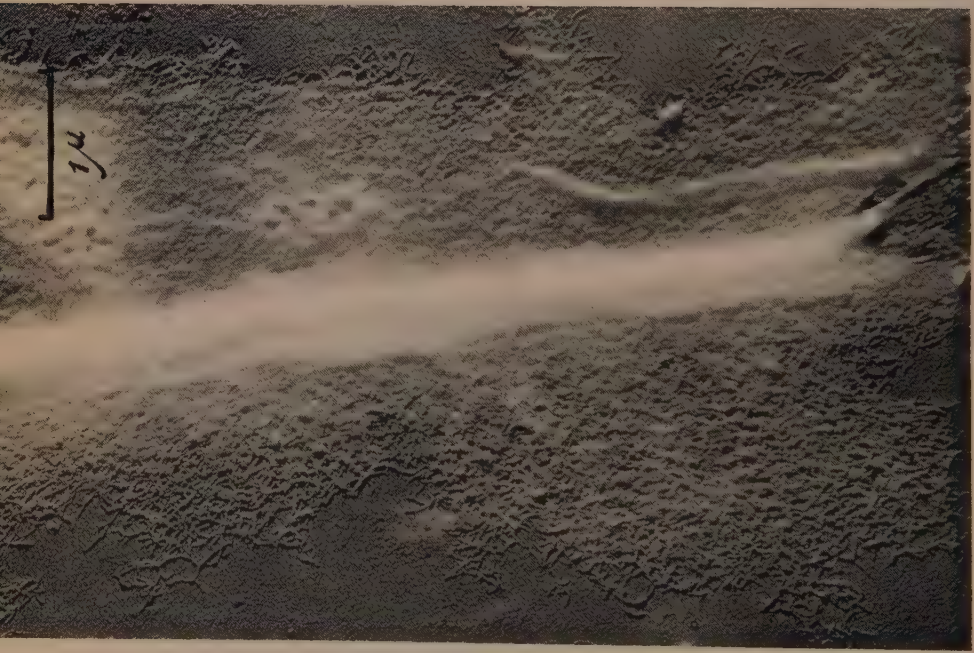

FIG. 6

P. A. ROELOFSEN AND D. R. KREGER—PLATE II

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DESCRIPTION OF PLATE I

Fig. 1. Ring diagrams from: (*a*) film, prepared from dissolved pectinic acid (4 per cent. methoxyl), irradiated perpendicular to its surface; (*b*) packet of unstretched pectin-skeletons from collenchyma bundles; (*c*) cellulose sphaero-crystals (cellulose II); (*d*) filter paper (cellulose I).

Fig. 2. X-ray diagram of slightly stretched pectin skeletons from collenchyma bundles in a vertical position. Note the slight equatorial intensification of the diffuse innermost reflection (PE) and the meridional accentuation of the reflection marked PM_1. (The reflection marked PM_2 is invisible on the reproduction.)

Fig. 3. Fibre diagram of fresh collenchyma bundles in air saturated with water vapour. PM_1 interference indicated.

Fig. 4. Fibre diagram of the same bundle as used for obtaining the diagram of Fig. 3, but without any precaution against drying out. PM_1, interference indicated.

Fig. 5. Cellulose fibre diagram obtained from collenchyma bundles freed from non-cellulose constituents. Cellulose (021) interference indicated.

The Absorption of Ions by Excised Root Systems

II. OBSERVATIONS ON ROOTS OF BARLEY GROWN IN SOLUTIONS DEFICIENT IN PHOSPHORUS, NITROGEN, OR POTASSIUM

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SUMMARY

1. Barley plants were grown in complete culture solution and in deficiencies of phosphorus, nitrogen, or potassium for a period of about 6 weeks. Excised roots of these plants were treated with a complete, aerated culture solution at 25° C. for varying periods of time, and the changes in respiration rate, phosphorus, nitrogen, potassium, sugars, and starch contents measured.

2. There were changes in fresh weight and dry weight of the excised roots during treatment. The dry weight decreased with time but the water-content changes were variable. There was a gain or loss of water by the roots according to the treatment.

3. In all cases the deficient roots increased in content of the element in which they were originally deficient. The roots of the plants supplied with full nutrient usually decreased in content of phosphorus, nitrogen, and potassium, but exceptions occurred and the reasons are discussed.

4. In most of the experiments described simultaneous loss of one ion and gain of another occurred.

5. Nitrogen-deficient roots accumulated nitrate when exposed to a complete nutrient solution, and some of this was assimilated with formation of protein. Under similar conditions nitrogen-rich roots decreased in nitrogen content and proteolysis took place.

6. There was a rapid fall in sucrose and reducing sugar content of the excised roots. The starch content was initially very small and showed little change with time.

7. The respiration rate declined with time in all treatments except where a nitrogen deficiency existed. Here the respiration rate increased to a maximum value at about 8 hours and then fell. This increase in rate is attributed to protein synthesis. No evidence of a 'salt respiration' was observed even when active uptake of phosphorus or potassium was occurring.

8. In most instances the carbon dioxide evolved in respiration greatly exceeded the carbon dioxide equivalent of the sugar consumed in the same period. Exceptions were found with the nitrogen-deficient roots where less carbon dioxide was evolved than the equivalent of sugar consumed. It is probable that a part, at least, of the sugar unaccounted for was used in protein synthesis.

9. Where the carbon dioxide of respiration was in excess of the equivalent of sugar consumed, protein or amino-acid is the most probable substrate. Respiration rate is found to be related both to nitrogen and sugar content.

Journ. of Experimental Botany, Vol. II, No. 6, pp. 344–379, September 1951.

10. Statistical examination of the data revealed that the gain or loss of an element showed a quadratic relationship with time. The rates of gain or loss of each element in each experiment were calculated at 0 hr. and 16 hrs. There was a negative correlation between rate and content of element and a positive correlation between rate and sugar content. Thus for a given sugar content there was a critical concentration of element above which loss occurred and below which gain occurred. Similarly for a given content of element there was a critical sugar content below which loss, and above which gain, of element occurred. The relative effect of one element on uptake of another was investigated and it was concluded that in most cases the uptake of any element was virtually independent of another.

11. The changes in water content of the excised roots was found to be related to the rate of potassium uptake in some instances.

12. The conclusions to be drawn from these experiments are discussed in the light of current theories. The present results do not support the theory of 'salt respiration' nor the alternative theory which postulates that active protein synthesis is a prerequisite of salt uptake. It was found that active uptake could occur simultaneously with marked proteolysis. The uptake observed could not be explained on the basis of ion exchange.

EXPERIMENTAL METHODS AND MATERIALS

WHEN excised roots of plants grown under varying conditions of nutrient supply are placed in a complete aerated culture solution, they show a net gain or loss of particular ions after a 24-hour period according to their previous nutritional history (Humphries, 1950). It was found that roots of plants grown under conditions of adequate nutrition invariably suffered a progressive loss of ions when excised and placed in the complete nutrient solution, while the excised roots of plants grown in solutions deficient in a particular ion absorbed that ion although loss of other ions usually occurred. These preliminary experiments suggested that the internal concentration of a given ion was one of the factors determining the rate of uptake of that ion and also that the carbohydrate status of the root had an influence on ion absorption. Further experiments were therefore planned, to be described in detail here, in which plants were grown in such a manner as to have widely differing nutrient and carbohydrate contents. Subsequently the ion uptake of the excised roots of these plants was observed over a 24-hour period.

Barley (var. 'Spratt Archer', strain BN8) was germinated on filter-paper in glass vessels for 2 days at room temperature when the roots were about 1–2 cm. long, and then placed on strips of waxed netting which was supported on wooden grids over tanks (Humphries, loc. cit.) of distilled water. There were 6 plants on each strip and 12 strips per tank. After a few days, when the plant had become established, 6 tanks were supplied with complete nutrient (half-strength Hoagland solution), and in the first experiment 6 tanks with complete nitrogen and potassium but only half the amount of phosphorus, and 6 tanks with only one-sixth the amount of phosphorus (see Table I). Each tank was supplied with ferrous tartrate and Hoagland's A4 solution. In this and the subsequent experiments no renewal of the nutrient solution was made during the growth period. The tanks were arranged in a row of 6 randomized blocks. This constituted one-half of the experiment and a duplicate set of 18 tanks were set up exactly 1 week later. This was to facilitate sampling as it was not possible to harvest all the samples and give them appropriate treatment at one time. Four days prior to sampling, half the tanks of each treatment, i.e. 9 tanks altogether, were darkened by covering with light-tight shades to provide plants with a lower carbohydrate content.

The plants were sampled by selecting at random 2 strips from each of the 3 tanks of each unshaded treatment, giving 36 plants per sample. Thus it was possible to obtain 6 samples from each treatment. The excised roots from these plants furnished the initial sample (0 hr.) and 5 samples for treatment with a flowing, aerated, complete culture solution (*full-strength* Hoagland solution) at 25° C. for the following periods of time: 2, 4, 8, 16, and 24 hours. A similar set of samples from the darkened plants were collected and treated the following day; the growth period of these plants was actually a day longer than that of the unshaded plants, but this will not materially affect the conclusions. For details of the apparatus in which the excised roots were treated see Humphries (loc. cit.).

Before treatment each sample of excised roots was washed in distilled water, centrifuged for 2 minutes at 2,000 rev. per min. to remove surface water, and the initial fresh weight obtained. The roots were similarly treated at the end of the experimental period to obtain the final fresh weight.

It was not found possible to handle all 6 samples of each treatment at once, nor to accommodate them in the thermostat together, so that sampling had to be staggered over a period of time. Thus it is possible that the initial composition of the samples was influenced by diurnal changes both of mineral and carbohydrate content, but no evidence of this was discovered. The order of collection of the samples was determined by the period of duration of the treatment so that samples could be removed from the apparatus at a convenient time; the following times of collection were adhered to:

8–hr.	sample	9.00 a.m.
4–hr.	,,	9.30 a.m.
24–hr.	,,	10.00 a.m.
0–hr.	,,	11.30 a.m.
2–hr.	,,	2.00 p.m.
16–hr.	,,	5.00 p.m.

The corresponding samples of each of the three treatments were collected at the same time.

Each sample in this experiment (subsequently referred to as the *phosphorus experiment*) was, after treatment, divided into approximately equal portions and one portion refluxed in alcohol for carbohydrate analysis while the other was oven-dried for mineral analysis.

A similar experiment was performed with nitrogen-deficient plants, but in this experiment the three treatments were (1) complete, (2) $\frac{1}{6}$ N, and (3) $\frac{1}{30}$ N, to give greater deficiencies than obtained in the phosphorus experiment. A third experiment was performed with three levels of potassium. For details of the composition of the nutrient solutions applied in these experiments see Table I. In these two experiments carbohydrate determinations were made on the 0-hour and 16-hour samples only.

TABLE I

Composition and concentration (g. mol. per l.) of culture solutions used

		Complete	$\frac{1}{2}$ P	$\frac{1}{6}$ P
Phosphorus experiment	KNO_3	0·0025	0·0025	0·0025
	$Ca(NO_3)_2$	0·0025	0·0025	0·0025
	$MgSO_4$	0·001	0·001	0·001
	KH_2PO_4	0·0005	0·00025	0·00008
	KCl	—	0·00025	0·00042

		Complete	$\frac{1}{6}$ N	$\frac{1}{30}$ N
Nitrogen experiment	KNO_3	0·0025	0·00042	0·00008
	$Ca(NO_3)_2$	0·0025	0·00042	0·00008
	$MgSO_4$	0·001	0·001	0·001
	KH_2PO_4	0·0005	0·0005	0·0005
	KCl	—	0·00208	0·00242
	$CaCl_2$	—	0·00208	0·00242

		Complete	$\frac{1}{6}$ K	$\frac{1}{30}$ K
Potassium experiment	KNO_3	0·0025	0·00042	0·00008
	$Ca(NO_3)_2$	0·0025	0·00354	0·00371
	$MgSO_4$	0·001	0·001	0·001
	KH_2PO_4	0·0005	0·00008	0·00002
	NaH_2PO_4	—	0·00042	0·00049

Respiration rate also was determined on some samples in each experiment. In the phosphorus experiment the respiration rate of the 24-hour samples of the complete and $\frac{1}{6}$ P was determined over 3-hourly periods. In the other two experiments the respiration rate of the 16-hour samples of the complete and $\frac{1}{30}$ treatments were determined over 2-hour periods using the Blackman commutator. The CO_2 liberated was absorbed in barium hydroxide solution containing barium chloride in Pettenkofer tubes. The culture solution issuing from the samples was separated from the gas-stream by means of the arrangement shown in Fig. 1. The stream of air and culture solution issuing from the root vessel passed through tube A into a glass vessel B where the solution fell to the bottom and finally siphoned to waste, while the air passed through tube C to the respiration apparatus. The culture solution used in these experiments had previously been rendered CO_2-free by vigorously bubbling a stream of CO_2-free air through it for a period of 2 hours. It was necessary to aerate the respiration samples with a fairly rapid stream of CO_2-free air (about 20 l. per hour) in order to carry over the culture solution, but by employing 0·2N baryta there was no evidence of CO_2 escaping absorption in the Pettenkofer tubes. The rate of flow of culture solution to each root container was about 0·2 litres per hour, which ensured complete renewal every $2\frac{1}{2}$ hours.

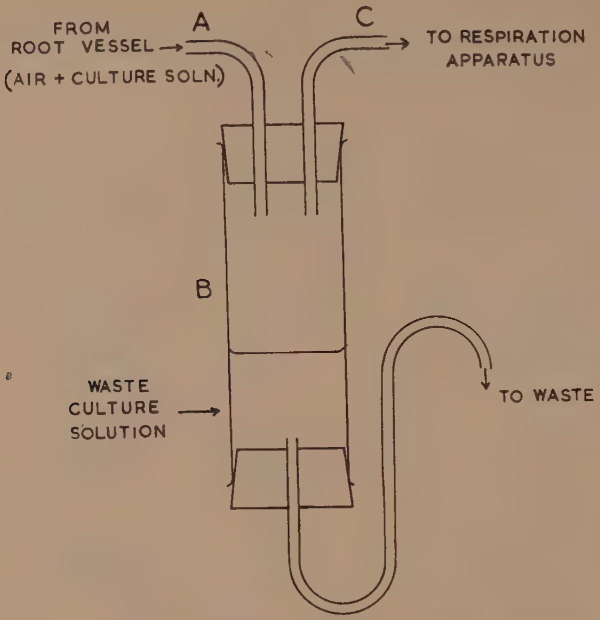

FIG. 1. Device for separation of air and culture solution. For explanation see text.

ANALYTICAL METHODS

Potassium, nitrogen, and phosphorus were determined by the methods already cited (Humphries, loc. cit.). Nitrate was determined on the dried material by heating 50-mg. samples (after frothing ceased) with 2·5 ml. of 20 per cent. sulphuric acid with approximately 0·3 g. of reduced iron in micro-kjeldahl digestion tubes over a low flame for about 5 minutes and distilling in a Markham apparatus in the usual way. Controls without reduced iron were also set up. One drop of liquid paraffin prevented excessive frothing during distillation. Nitrate-nitrogen was estimated by the excess ammonia produced by iron reduction. This simple procedure was found to give accurate and reproducible results.

Sucrose and reducing sugars were determined by the method of Harding and Downs as modified by Van der Plank (1936). Starch values on the alcohol insoluble material were determined by hydrolysis with β-amylase by the method described by Hanes (1936).

EXPERIMENTAL RESULTS

I. *The effect of cultural treatment on yield of tops and roots*

A. *Phosphorus experiment*

In Table II are presented the fresh and dry weights of the tops and the fresh weights of the excised roots before treatment. Each weight is the mean of 12 samples. It is not possible to give comparable figures for the initial dry weights of the roots since all samples are subject to variable treatments. The effect of 4 days' complete darkness was to reduce the fresh weight of both tops and roots; the dry weight of the tops was also decreased. The fresh weight of the roots in the $\frac{1}{2}$ P treatment was higher than in the complete treatment. The effect of a greater phosphorus deficiency ($\frac{1}{6}$ P) was to produce a still larger root weight. This is a well-known effect of phosphorus deficiency on the size of the root system. The greatest phosphorus deficiency decreased both fresh weight and dry weight of tops.

B. *Nitrogen experiment*

The plants for this experiment were grown at a more favourable time of the year and the fresh weights per plant were higher, although this is partly attributable to a longer growth-period (see Table II). Exactly the same amounts of nutrients were supplied in the 'complete' treatment in each experiment, so that the weight per plant from this treatment is a rough guide to the growth conditions experienced by the plants in each experiment. The effect of a moderate nitrogen deficiency ($\frac{1}{6}$ N) was to reduce the fresh weight of the tops much more markedly than that of the roots—in fact the root weight of the unshaded plants in this treatment was greater than that of the shaded plants in the complete treatment. In the lowest nitrogen treatment ($\frac{1}{30}$ N) the root fresh weight was much reduced but shading had no effect. The fresh weight of the tops was reduced by nitrogen deficiency, but the fresh weights of the shaded samples in both $\frac{1}{6}$ N and $\frac{1}{30}$ N were larger than those of the corresponding unshaded samples. This is due to a greater water content, as may be seen from the dry-weight figures in the adjoining columns (Table II). In both, the dry weight of the shaded is less than that of the unshaded samples.

TABLE II

Yield of tops and roots of barley plants grown in water culture

	Fresh weight (g.) per 36 plants				Dry weight (g.) per 36 plants	
	Roots		Tops		Tops	
Time of year when plants were grown	Unshaded	Shaded	Unshaded	Shaded	Unshaded	Shaded
Complete Sept.–Oct.	16·06	14·60	52·8	49·5	4·2	3·5
$\frac{1}{2}$ P	16·32	13·09	54·9	47·8	4·2	3·2
$\frac{1}{6}$ P	20·96	17·40	50·7	46·5	4·0	3·2
Least significant difference	6·47		14·3		0·9	
Complete Jan.–Mar.	29·75	21·92	84·1	78·3	7·0	5·5
$\frac{1}{6}$ N	27·97	25·16	40·4	48·6	4·0	3·5
$\frac{1}{30}$ N	13·67	13·91	15·8	18·8	1·7	1·6
L.S.D.	9·58		14·9		1·2	
Complete Mar.–May	60·16	45·82	108·9	108·8	10·7	8·4
$\frac{1}{6}$ K	46·80	36·64	80·2	80·0	8·8	7·0
$\frac{1}{30}$ K	25·20	22·11	50·9	51·5	6·1	5·1
	12·45		28·7		2·7	

C. *Potassium experiment*

Here the plants in the complete treatment were the largest of the three experiments and the shading treatment caused appreciable decrease in root fresh weight, which is statistically significant. There was virtually no difference in fresh weight of the tops as a result of shading in any of the three treatments, but the dry weights of the shaded plants were lower, so that here also the percentage water content was increased by shade. It will be noticed from Table II that potassium deficiency caused less reduction in dry weight than did nitrogen deficiency, although a strict comparison between the two experiments is not permissible since they were performed in different seasons.

II. *The effect on fresh weight of excised roots when exposed to an aerated, complete culture solution*

In order to estimate the relative parts played by water content and dry matter in the changes of fresh weight during treatment the final fresh weight has been expressed as a percentage of the value before treatment, and, by subtracting the dry matter expressed as a percentage of the fresh weight before treatment, the changes in water content, as a percentage of fresh weight before treatment, can readily be followed. In all three experiments there was a progressive loss of dry matter (see Fig. 2) except in the $\frac{1}{30}$ N treatment, both shaded and unshaded, where the dry matter increased during the first 8 hours. No explanation is forthcoming at the moment for this rather curious result.

There are two main causes of loss of dry matter of excised roots when subjected to the experimental conditions. The first, which probably accounts for the bulk of the loss, is due to breakdown of respiratory substrate. The second is loss of mineral substances from the root. There is no means whereby the dry-matter content of the excised root could increase appreciably except by absorption of ions.

The water-content changes, however, were variable in the different experiments. It is worth noting at this point that the fresh weights after treatment may be slightly underestimated since some mechanical loss of root fragments is likely to occur in transferring the roots from the glass vessels into the centrifuge basket and then into the weighing tins. Hence the gain of water in these experiments is likely to be slightly underestimated in some cases.

In the phosphorus experiment the roots decreased in fresh weight with time, but in the nitrogen experiment there was a general tendency for the unshaded samples to increase in water content and this increase was greater than the decreases of dry weight, so that during treatment the fresh weight rose to over 100 per cent. of the initial (0-hour) value in some cases. In the shaded samples the water content declined. There was a similar state of affairs in the potassium experiment where with unshaded treatments the roots increased and with shaded treatments decreased in water content.

As pointed out earlier, the 'complete' treatments in all three experiments were identical, but there was a difference in behaviour of the excised roots of

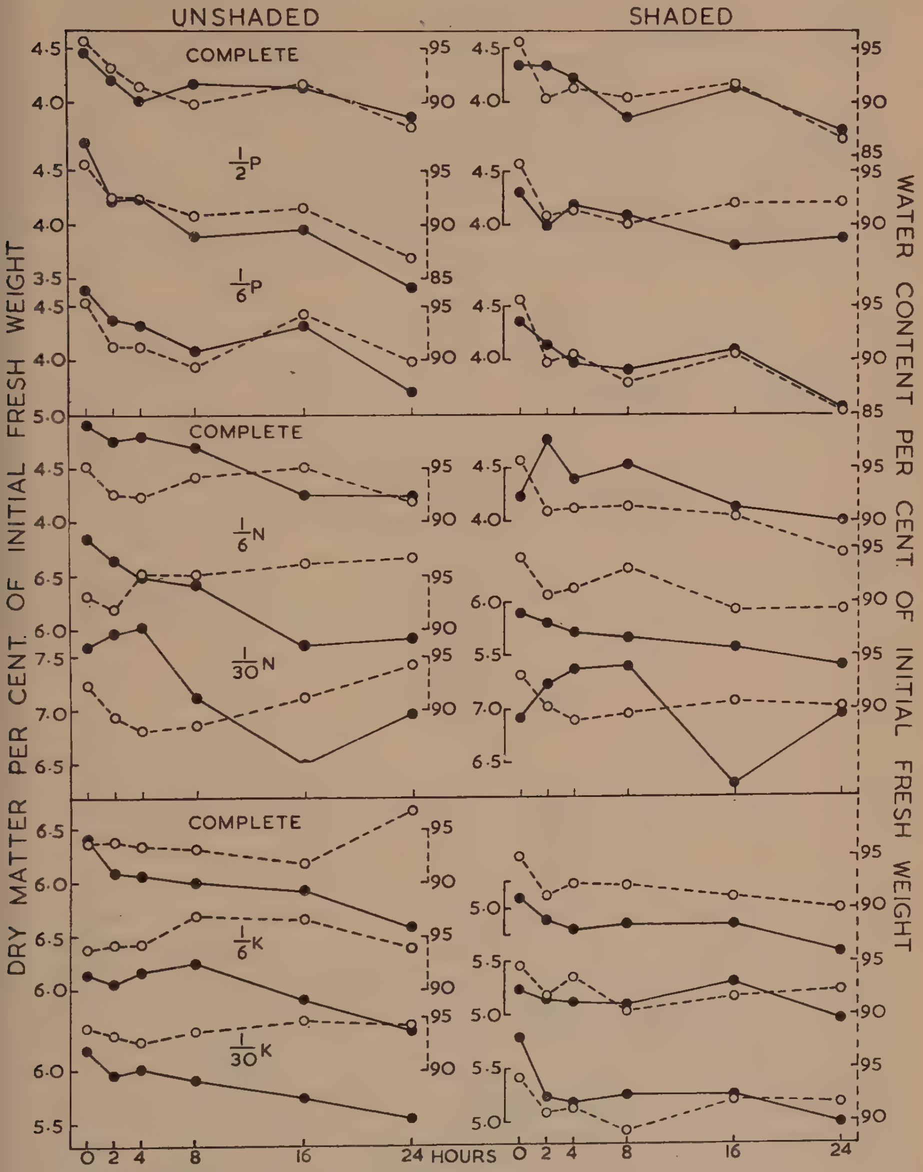

FIG. 2. The changes in dry matter and water content of excised roots of barley plants, grown under varying nutrient conditions, during the course of 24 hours.

●———● dry matter ○- - - - -○ water content

this treatment in these experiments, and this is apparently related to the season in which they were grown. The seasonal factor concerned appears at first sight to be carbohydrate. Excised roots with a fairly high initial carbohydrate content tend to gain water. In the shaded series there was a loss of water in most cases. The possible relationship between water gain or loss and carbohydrate content will be discussed in a later section.

III. *Treatment effects and interactions*

The data obtained in the three experiments were subjected to an analysis of variance and the results will be briefly summarized here in order to give an idea of the differences in content brought about by the different nutrient treatments to which the plants were subjected during growth and the subsequent treatment of the excised roots. The nitrogen, phosphorus, and potassium contents of each sample in the three experiments were determined and and in each experiment there were 6 sampling times at each of the 3 levels of nutrition, which in turn were either shaded or unshaded and each treatment was duplicated. There are therefore 72 analyses for each of the three elements in each experiment. The analysis of variance was divided into two parts, the first accounting for the variance due to the different conditions of growth, and the second for treatment changes. The significant effects and interactions and their probable levels of significance are shown in Table III. In the phosphorus experiment the level of phosphorus in the roots alone was significantly affected by the growth conditions, while in the nitrogen experiment the varying treatments affected significantly the phosphorus contents as well as the nitrogen contents, and in the potassium experiment the contents of all three elements were influenced by the different potassium treatments. Shading affected the levels of nitrogen and potassium, but not of phosphorus, in all three experiments. There was an interaction between phosphorus content

TABLE III

Significant effects and interactions of nutrient content with time and shade

	Nutrient content (N, P, or K)	S	Nutrient ×S	T	Nutrient ×T	T×S	Nutrient ×S×T
			Phosphorus experiment				
P	0·001	—	0·001	0·05	0·001	—	—
N	—	0·001	—	0·001	—	—	—
K	—	0·05	—	0·001	—	—	—
			Nitrogen experiment				
P	0·01	—	—	0·001	—	—	—
N	0·001	0·05	—	0·001	0·001	—	0·05
K	—	0·01	0·05	0·01	0·01	—	—
			Potassium experiment				
P	0·001	—	—	0·001	0·01	0·01	—
N	0·001	0·05	—	0·01	0·01	0·001	—
K	0·001	0·001	—	0·001	0·001	0·001	—

P = Phosphorus
N = Nitrogen
K = Potassium
S = Shade
T = Time

and shade in the phosphorus experiment and between potassium and shade in the nitrogen experiment. There was a significant drift with time of all three elements in all three experiments. There was a significant interaction between time and phosphorus in the phosphorus experiment, between time and nitrogen, time and potassium in the nitrogen experiment and between time and all three elements in the potassium experiment. The interaction between time and shading was only significant in the potassium experiment and here the effect was significant in the case of each of the three elements. There was only one case of a triple interaction being significant and that was in the case of time, nitrogen, and shade in the nitrogen experiment.

IV. *Changes in phosphorus, nitrogen, and potassium of the excised roots*

Since a change in fresh weight of the excised roots occurred as a result of treatment, the results of the analyses have been expressed as a percentage of the fresh weight before commencement of treatment, i.e. as a percentage of initial fresh weight. The changes in phosphorus, nitrogen, and potassium occurring in the three experiments have been plotted in Figs. 3, 4, and 6. The actual experimental data (means of duplicate samples) have been plotted, but the curves through the points have been calculated, as will be explained later.

A. *Phosphorus experiment*

The changes in phosphorus content of the excised roots of plants grown in complete nutrient solution are shown in Fig. 3. There was a steady loss of phosphorus from the roots of both shaded and unshaded samples. The trend in the $\frac{1}{2}$ P series was very similar, but in the $\frac{1}{6}$ P samples, where apparently a phosphorus deficiency had been induced, the unshaded samples increased in phosphorus content steadily for the first 8 hours. The shaded samples of this treatment also increased in phosphorus content, but to a smaller extent.

The changes in nitrogen content showed a very similar trend with all treatments. There was an appreciable loss of nitrogen after 24 hours, amounting, on the average, to about 30 per cent. of the original content. There appeared to be very little difference between the behaviour with time of shaded and unshaded samples, although the latter had a consistently higher N content. There was also a considerable loss of potassium in all samples—an average of 37 per cent. of the original amount. Thus in the $\frac{1}{6}$ P treatment there was a gain of phosphorus and a simultaneous loss of potassium and nitrogen.

B. *Nitrogen experiment*

There was a consistent loss of phosphorus in all treatments in this experiment.

In the complete unshaded treatment there was a steady loss of nitrogen and the shaded treatment where the initial nitrogen content was lower showed

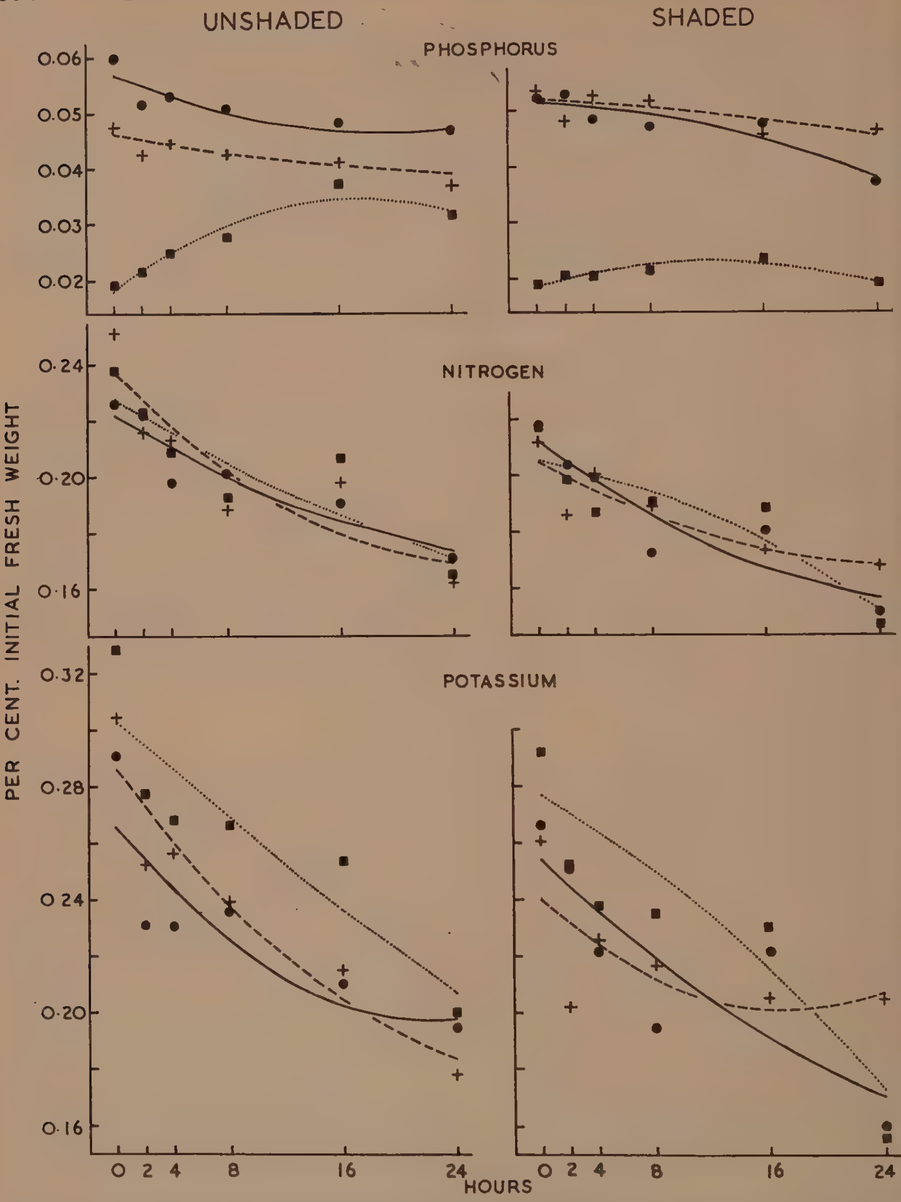

FIG. 3. The changes in phosphorus, nitrogen, and potassium content of excised roots of barley grown under varying conditions of *phosphorus* supply when subjected to an aerated complete culture solution.

●——— complete, +------$\frac{1}{2}$P, ■······$\frac{1}{6}$P

a similar trend (Fig. 4). In the medium nitrogen treatment ($\frac{1}{6}$ N) the initial nitrogen content was reduced to about half that of the complete treatment, and in both the unshaded and shaded treatments there was an increase in nitrogen content throughout the 24 hours, but the increase was less in the shaded samples. The $\frac{1}{30}$ N samples had a nitrogen content of about one-third that of the complete series. The roots of this series increased rapidly in nitrogen content; again the shaded samples showed a smaller increase than the unshaded. The nitrogen content of the latter reached a value after 24 hours similar to that to which the shaded complete treatment samples had fallen.

The potassium content in the complete treatment fell appreciably after 24 hours (about 30 per cent.), but there was not much net change in the potassium content of the unshaded $\frac{1}{6}$ N plants, but the shaded counterpart lost potassium. Both shaded and unshaded samples of the $\frac{1}{30}$ N treatment gained potassium. It may be seen from Fig. 4 that there was a relationship between the level of nitrogen applied to the plant and the potassium content as well as between potassium content and shade. Thus at the $\frac{1}{30}$ N treatment there was a considerably lower potassium content than at the other two levels of nitrogen. Shade decreased the potassium content except in the $\frac{1}{30}$ N treatment.

Again, in the experiment certain samples showed simultaneous loss and gain of ions—in this instance gain of nitrogen and loss of potassium and phosphorus.

In addition to total nitrogen, nitrate-nitrogen was also determined. There was a steady loss of nitrate from the roots of the plants grown in complete culture solution, amounting to about 0·01 per cent. nitrate-nitrogen on a fresh-weight basis (see Fig. 5). There was a loss of similar magnitude from the corresponding shaded samples, but the initial nitrate content was slightly lower than in the unshaded plants. The loss in nitrate-nitrogen was much smaller than the loss of total nitrogen during the course of the experiment (cf. Figs. 4 and 5). At both levels of nitrogen deficiency there was no nitrate present in the initial samples, but nitrate accumulated practically linearly during 24 hours, and at the end of this time there was approximately 0·04 per cent. nitrate-nitrogen present. At both levels of nitrogen deficiency the rates of accumulation of nitrate-nitrogen were similar. The accumulated nitrate accounted for about half the increase in the total nitrogen of these samples; hence it must be assumed that nitrate was assimilated. Nitrate also accumulated in the nitrogen-deficient roots of the shaded plants, but at a lightly lower rate than the unshaded plants. Here also there was evidence of assimilation of nitrate.

In the nitrogen experiment there was a considerable loss of nitrogen from the roots of plants grown in the complete series, but a considerable gain of nitrogen by the roots of plants grown under conditions of nitrogen deficiency. In order to obtain some idea of what fractions of the nitrogen were concerned in the losses obtained, and whether actual assimilation of the nitrate absorbed by the deficient plants occurred, a subsidiary experiment was set up, some plants being supplied with complete nutrient and others with $\frac{1}{30}$th of the nitrogen of the complete solution. The plants were sampled in the usual way after 42 days' growth and the roots excised and placed in complete aerated flowing culture solution at 25° C. for periods of 8, 16, and 24 hours. The samples, after treatment for the appropriate time, were centrifuged, weighed,

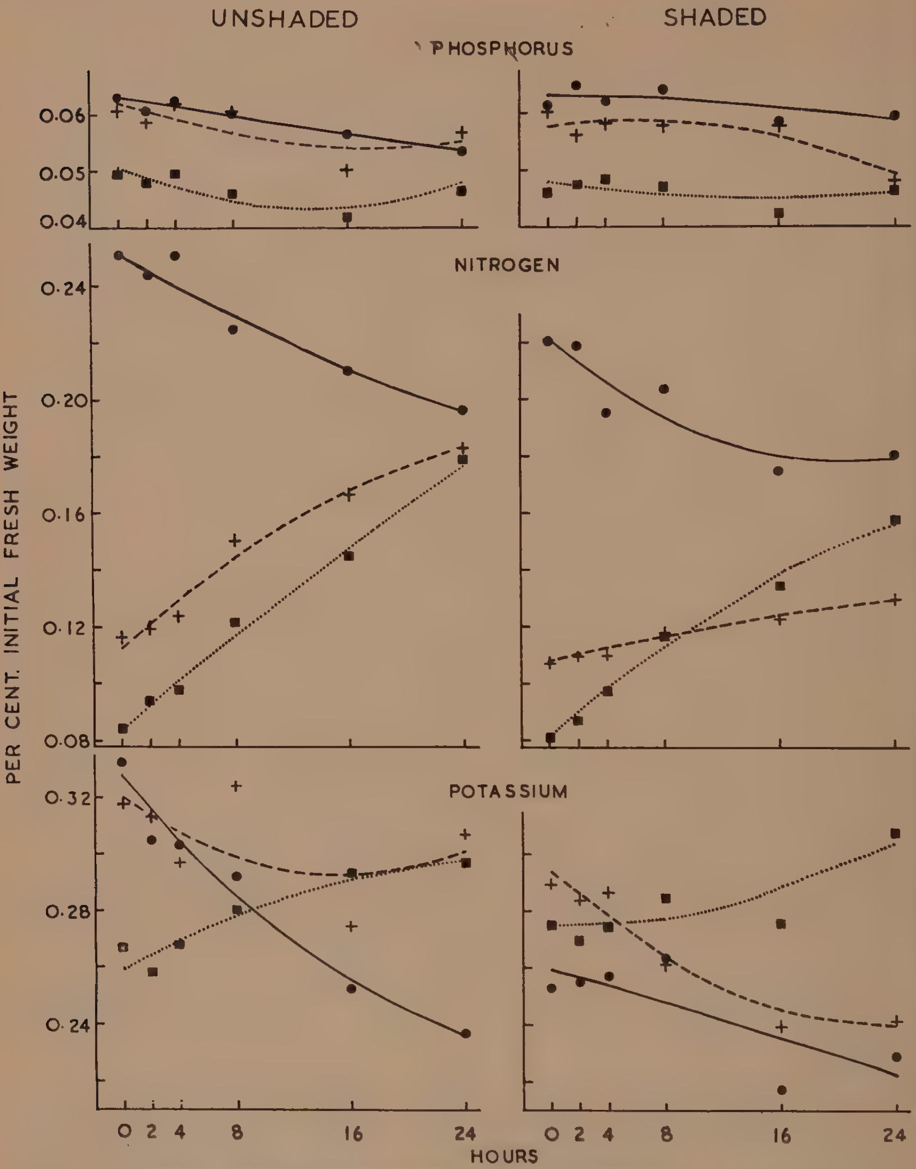

FIG. 4. The changes in phosphorus, nitrogen, and potassium content of excised roots of barley grown under varying conditions of *nitrogen* supply when subjected to an aerated complete culture solution.

●——— complete, +------$\frac{1}{8}$N, ■······$\frac{1}{30}$N

and then ground up with the calculated amount of 10 per cent. trichloracetic acid to make the final concentration of acid 5 per cent. The preparations were then filtered and the filtrate made to volume with 5 per cent. trichloracetic acid. Total nitrogen and nitrate determinations were made on the filtrate, and the whole residue was digested with sulphuric acid (as it was not practicable to take a representative sub-sample) and an aliquot of the digest distilled in the usual way to obtain the total nitrogen content of the residue. The experiment was replicated, and the mean values of total soluble N, nitrate N, and total N of residue ('protein' N) are presented in Table IV. The soluble N of the roots of plants grown in complete solution tended to increase somewhat during treatment while the nitrate shows a slight loss; the latter is in agreement with the results for nitrate determinations made on the dry material

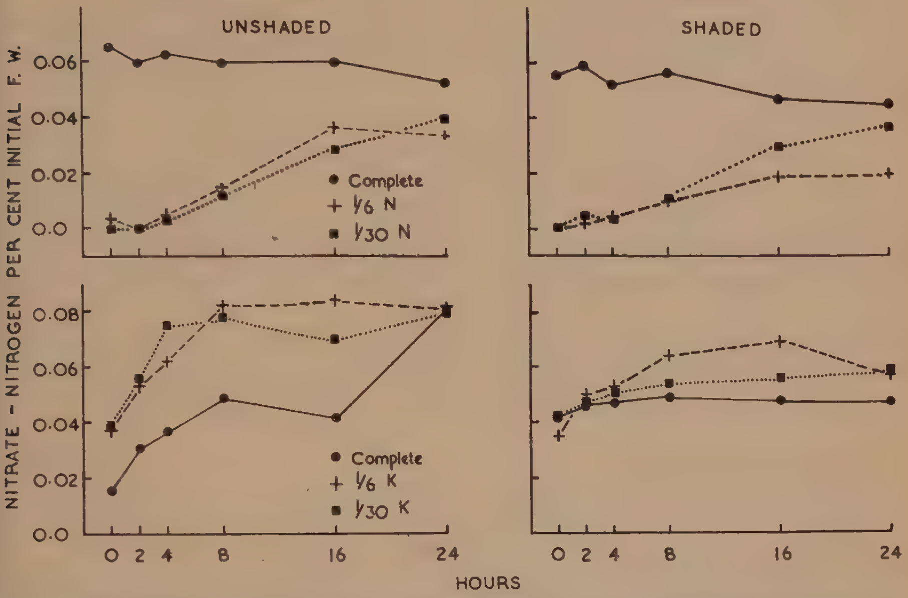

FIG. 5. The changes in nitrate-nitrogen content of excised roots of barley grown under varying conditions of nitrogen and of potassium supply when subjected to an aerated complete culture solution.

TABLE IV

Changes in the protein and soluble nitrogen of excised roots of barley grown in complete and in nitrogen-deficient cultures when immersed in a complete aerated culture solution

Treatment	Time hrs.	'Protein'-N	Soluble-N	Nitrate-N	Total-N	Respiration rate mg. CO_2/g. F.W./hr
		% F.W.				
Complete nutrient	0	0·156	0·082	0·044	0·238	
	8	0·149	0·096	0·042	0·245	
	16	0·139	0·087	0·040	0·226	
	24	0·122	0·101	0·040	0·223	
$\frac{1}{30}$ N	0	0·085	0·013	0·001	0·098	0·278
	8	0·106	0·039	0·012	0·143	0·595
	16	0·102	0·058	0·019	0·160	0·345
	24	0·104	0·057	0·025	0·161	

from the nitrogen experiment (p. 355). The insoluble or protein N, on the other hand, decreased during treatment from an initial value of about 0·16 to 0·12 after 24 hours. It thus appears that the protein N becomes broken down into a soluble form which is subsequently lost from the roots. The form in which the nitrogen is lost was not investigated but is possibly amino acid or amide. It does not appear to be lost as nitrate, since the nitrate concentrations alters but little, unless nitrate is continuously formed from the breakdown products of protein.

The deficient roots, on the other hand, increase rapidly in content of both nitrate and soluble N, the latter increasing at a greater rate than the former (Table IV). There is also an increase in insoluble or protein N during the first 8 hours, after which the amount remains practically constant. Thus excised, nitrogen-deficient roots exposed to a complete culture solution absorb nitrate which not only accumulates as such but is also assimilated and converted to protein nitrogen.

Synthesis of protein from nitrate by isolated root systems of oats has been demonstrated by Postma (1939), and Burström (1937, 1939, 1940) has quantitatively determined the nitrate consumption of isolated wheat roots.

C. *Potassium experiment*

There was a small increase in phosphorus content with the complete unshaded treatment and a small loss with the shaded treatment (Fig. 6). The $\frac{1}{6}$ K samples behaved somewhat similarly with a small increase in the shaded samples and a small loss in the unshaded. Both unshaded and shaded samples of the $\frac{1}{30}$ K lost phosphorus during treatment. The shaded samples of each treatment had a greater initial phosphorus content than the corresponding unshaded samples.

The potassium treatments had a big effect on the initial nitrogen contents of the roots—the nitrogen content increasing with increasing deficiency. The unshaded samples with all three treatments increased in nitrogen content during treatment while all the shaded samples declined. There was a tendency for the unshaded samples to reach a maximum nitrogen content at about 8 hours, subsequently falling.

The unshaded samples with the complete treatment increased in potassium, reaching a maximum after 8 hours. The shaded samples with the complete treatment lost potassium. There was a very rapid uptake of potassium with the $\frac{1}{6}$ K unshaded treatment—a fourfold increase in 8 hours when the maximum content was attained. The shaded samples with this treatment also increased in potassium content but at a much smaller rate. The unshaded $\frac{1}{30}$ K samples also showed a threefold increase in potassium content in 8 hours, while the shaded samples also increased in potassium but to a smaller extent.

There is a noticeable difference in behaviour of the roots in the complete treatment in this experiment from that in the complete treatments in the other two experiments, and this is undoubtedly due to the fact that the plants were much larger so that the culture solution in which they were growing had

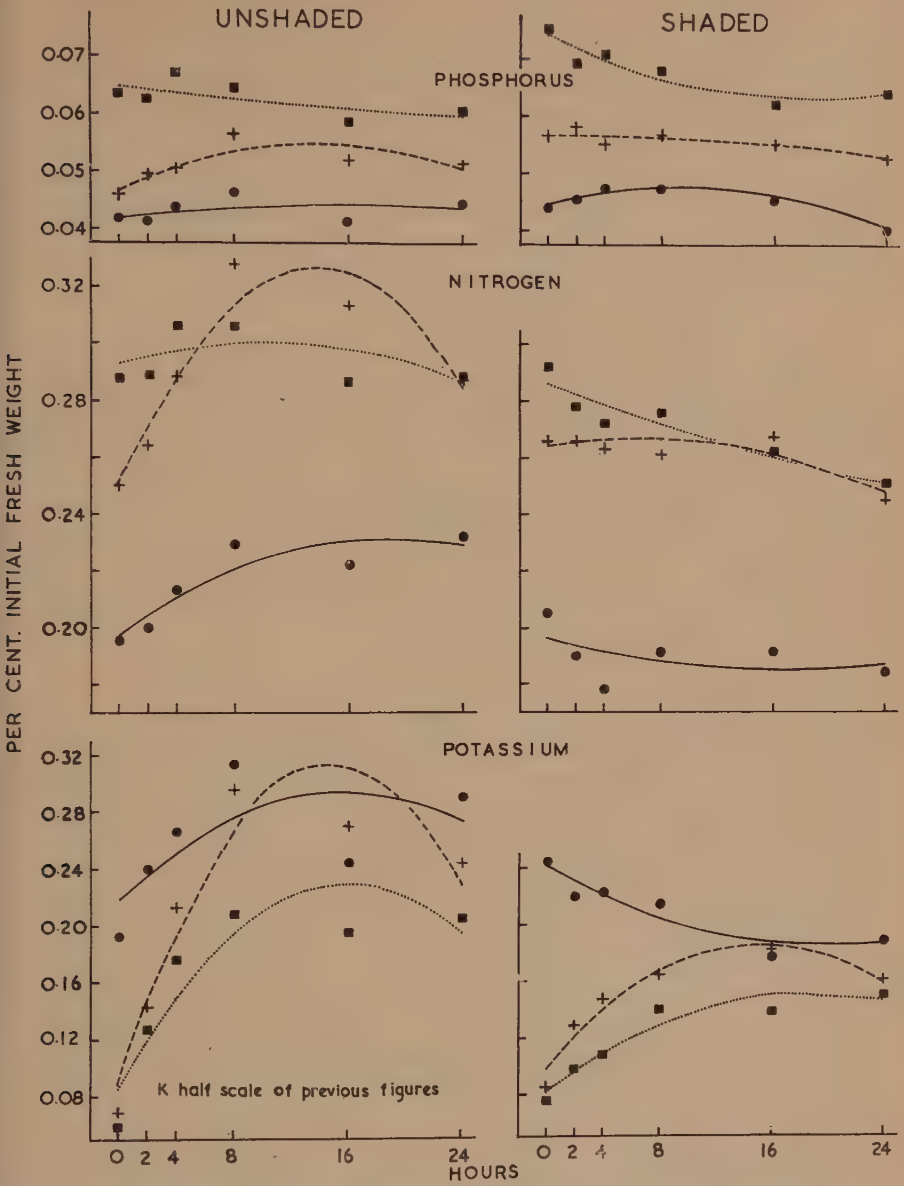

Fig. 6. The changes in phosphorus, nitrogen, and potassium content of excised roots of barley grown under varying conditions of *potassium* supply when subjected to an aerated complete culture solution.

●———— complete, +------$\frac{1}{8}$K, ■······$\frac{1}{30}$K

become exhausted and deficiencies existed at the time of harvesting. This accounts for the uptake of phosphorus, nitrogen, and potassium by the unshaded samples.

The drift in nitrate content was also investigated. In all three levels of potassium the unshaded samples increased in nitrate content during the experiment to reach a value after 24 hours of about 0·08 per cent. (Fig. 5). Comparison of this increase with the increase in total nitrogen (Fig. 6) shows that some of the nitrogen absorbed accumulated as nitrate, but the increase of the latter did not account for the total increase in total N, i.e. the fraction total N$-NO_3$–N increased. This point will be returned to later when the rates of uptake are considered. Thus, here is a case of roots already high in nitrogen increasing still more when placed in a complete culture solution, but it seems to be a case of uptake of NO_3 which is not readily utilized.

V. *Changes in carbohydrate content of the excised roots*

Estimation of soluble carbohydrates (sucrose and total reducing sugars) and of starch were made on certain samples. The amount of starch present was extremely small—of the order of 0·01 per cent. of fresh weight, and showed little change during the course of the experiment. In the majority of cases the extract from the alcohol-insoluble residue gave no blue colour with iodine. It was concluded that there was no considerable reserve of starch in the barley roots used in these experiments which might give rise to sugars during the course of the experiment. In the following account, therefore, only changes in sucrose and reducing sugars are considered.

A. *Phosphorus experiment*

In this experiment carbohydrate determinations were done on all samples, viz. initial (0 hr.) and after 2, 4, 8, 16, and 24 hours exposure of the excised roots to the culture solution. The results are shown in Table V. The carbohydrate content of these plants, which were grown in September and October for a period of 32 days, is very low, and as the total amount of material available for analysis was small the carbohydrate figures are possibly not so reliable as in later experiments. It will be observed that the sucrose and reducing sugar content of the shaded samples was lower than that of those without shade. The sucrose content fell with time, the decrease being very marked between 0 and 2 hours in the unshaded series. There was not such a marked fall of reducing sugar with time, but in all samples, even the initial, the content of reducing sugars is very low.

B. *Nitrogen experiment*

This was performed at a more favourable time of the year for growth, and the plants, both tops and roots, had a higher carbohydrate content. Only the 0-hour and 16-hour samples were analysed for carbohydrate (see Table V). The effect of depriving the plants of nitrogen is reflected in the high sugar content (both sucrose and reducing sugars) of these plants, the $\frac{1}{30}$ N having over 1 per cent. of sucrose. In fact the absolute amounts of sucrose (g. per 36 plants) in the complete unshaded and complete shaded plants were 0·023 and 0·0007 respectively, while the corresponding figures for the $\frac{1}{30}$ N treatment

TABLE V

Sucrose and reducing sugar content of excised roots, as a percentage of initial fresh weight, of barley plants grown under conditions of varied nutrient supply and subjected to a complete aerated culture solution for different periods of time

	Complete nutrient				$\frac{1}{2}$ phosphorus				$\frac{1}{6}$ phosphorus			
	Unshaded		Shaded		Unshaded		Shaded		Unshaded		Shaded	
Hours	Sucrose	R.S.	Sucrose	R.S.	Sucrose	R.S.	Sucrose	R.S.	Sucrose	R.S.	Sucrose	R.S.
0	0·040	0·015	0·002	0·009	0·033	0·014	0·002	0·011	0·039	0·029	0·002	0·007
2	0·004	0·012	0·002	0·007	0·006	0·011	0·002	0·008	0·012	0·012	0·001	0·008
4	0·003	0·010	0·003	0·007	0·003	0·007	0·003	0·005	0·006	0·010	0·004	0·010
8	0·003	0·007	0·003	0·013	0·007	0·007	0·001	0·010	0·004	0·009	0·001	0·015
16	0	0·010	0	0·013	0	0·011	0	0·014	0	0·013	0·001	0·015
24	0·02	0·009	0	0·012	0·010	0·010	0·001	0·010	0	0·010	0·001	0·015
	Complete nutrient				$\frac{1}{6}$ nitrogen				$\frac{1}{30}$ nitrogen			
0	0·076	0·043	0·003	0·010	0·996	0·156	0·232	0·056	1·070	0·257	0·871	0·095
16	0·003	0·017	0·005	0·013	0·132	0·070	0·028	0·025	0·216	0·126	0·112	0·094
	Complete nutrient				$\frac{1}{6}$ potassium				$\frac{1}{30}$ potassium			
0	0·361	0·693	0·017	0·031	0·311	1·046	0·066	0·149	0·151	0·466	0·046	0·102
16	0·058	0·233	0·010	0·008	0·068	0·213	0·018	0·026	0·036	0·117	0·021	0·024

were 0·146 and 0·121. Thus the much smaller nitrogen-deficient plants had a greater absolute sugar content than those grown in complete nutrient.

After the excised roots had been exposed to culture solution for 16 hours there was a considerable reduction in sugar content.

C. *Potassium experiment*

The plants with the complete treatment had a higher carbohydrate content than those with the complete treatment in the nitrogen experiment (Table V). In all treatments the reducing sugar values tended to be greater than the sucrose values, in contrast to the results in the other experiments. Again there was a marked reduction in sugar as a result of shading and there was a rapid decrease in sugar content during treatment. The sucrose content decreased with increasing potassium deficiency, but the $\frac{1}{6}$ K treatment gave the highest reducing sugar content.

VI. *Changes in respiration rate of the excised roots*

The respiration rate has been expressed as mg. CO_2 per hour per g. initial fresh weight.

A. *Phosphorus experiment*

The drift in respiration rate over a 24-hour period is shown in Fig. 7. The initial rate of the complete unshaded sample was about 0·45 mg. CO_2 per hour, and this declined fairly rapidly at first and reached a fairly constant value of 0·15 mg. after 24 hours. The shaded sample of the complete treatment had a smaller initial respiration rate (0·26 mg. CO_2) which declined to about 0·11 mg. after 24 hours. Both the unshaded and shaded samples of the $\frac{1}{6}$ P series had a lower initial respiration rate than the corresponding samples of the complete treatment but reached approximately the same final value as these after 24 hours.

B. *Nitrogen experiment*

There was a marked contrast between the pattern of respiration in the complete and $\frac{1}{30}$ N treatments. Both the unshaded and shaded samples of the complete treatment showed a steady fall in respiration rate as in the case of the phosphorus experiment, but the general level of respiration was higher than in that experiment. As before, the shaded samples had a lower initial rate than the unshaded. The respiration rate of the $\frac{1}{30}$ N samples, both shaded and unshaded, increased from the beginning of the experiment to reach a maximum between 8 and 10 hours with a subsequent fall. The rate after 24 hours, however, was greater than the initial rate. The increase in respiration rate was greater in the unshaded samples. There is little doubt that the increase in respiration rate of the nitrogen-deficient roots is associated with the synthesis of nitrogenous compounds from the nitrate absorbed.

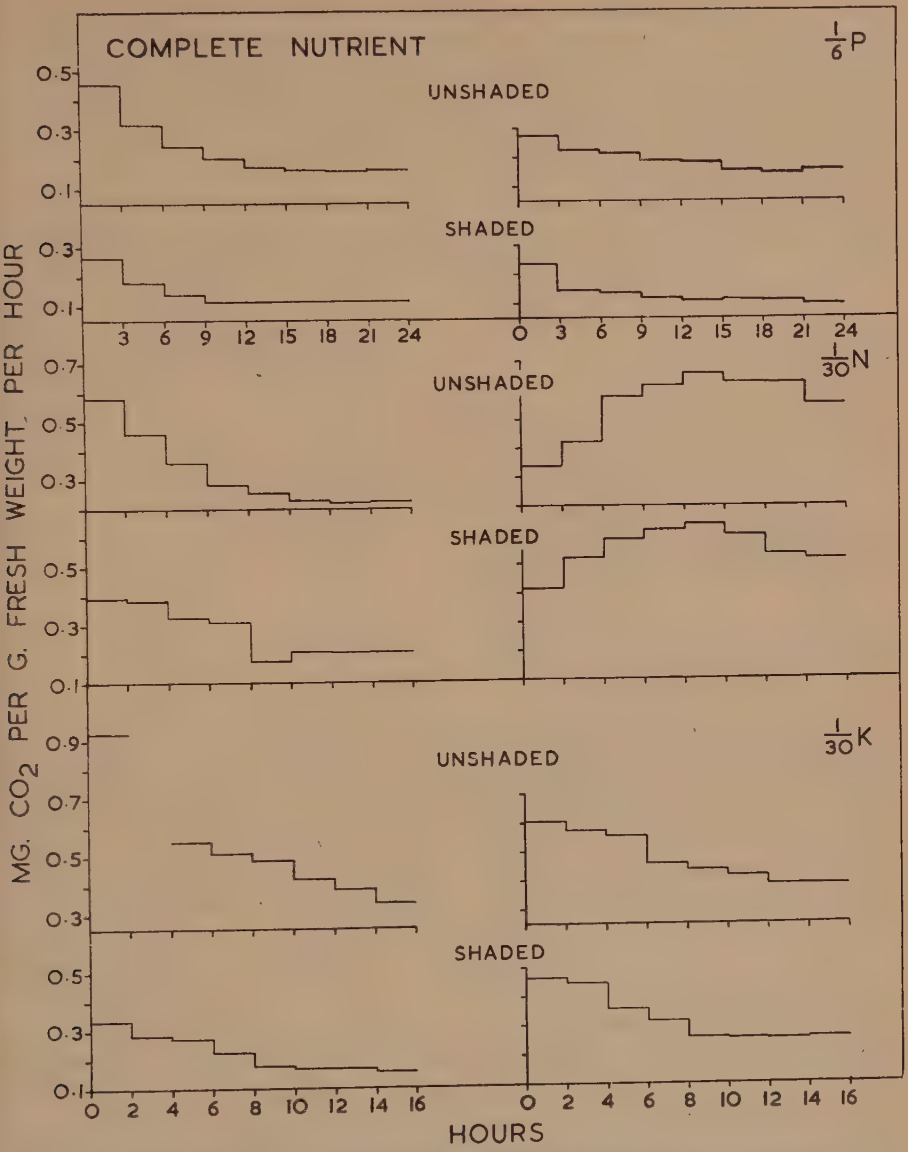

FIG. 7. The respiration rate of excised roots of barley plants grown under varying conditions of nutrient supply in a complete aerated culture solution.

C. *Potassium experiment*

In all treatments there was a decrease in respiration rate, the unshaded samples had a higher initial value than that of the shaded. The general level of respiration of the unshaded complete series was higher than that of the same treatment in the nitrogen experiment, which in turn was higher than that of the complete treatment of the phosphorus series.

In none of the experiments was there any evidence of a 'salt respiration' even when active entry of ions into the roots was taking place. The increase in respiration rate which occurred when nitrogen deficient roots were absorbing nitrogen is not strictly speaking a 'salt respiration' and is not due to the entry of ions but to their subsequent metabolism.

TABLE VI

Comparison of carbon dioxide evolved in respiration and carbon dioxide equivalent of sugar consumed during a period of 16 hours

		CO_2 evolved	CO_2 equivalent of sugar consumed
		mg. per 100 g. F.W.	
Phosphorus experiment	Complete unshaded	376	40
	,, shaded	234	3
	$\frac{1}{6}$ P unshaded	308	88
	,, shaded	208	2
Nitrogen experiment	Complete unshaded	523	151
	,, shaded	439	0
	$\frac{1}{30}$ N unshaded	880	1410
	,, shaded	881	1175
Potassium experiment	Complete unshaded	862	1143
	,, shaded	357	45
	$\frac{1}{30}$ K unshaded	762	690
	,, shaded	520	153

The changes in sugar concentration observed in the phosphorus experiment where determinations were made at 2, 4, 8, 16, and 24 hours indicate a big initial fall to a low level in the first few hours and a subsequent less rapid decline. It is assumed that the pattern of the carbohydrate changes are similar in the other experiments where estimations at 0 and 16 hours only are available. The decline in carbohydrate is presumably due mainly to respiratory oxidation, and it is of interest to compare the amount of sugar lost with the amount of carbon dioxide evolved over a 16-hour period (see Table VI). In all cases except three it is found that the carbon dioxide evolved in respiration is greatly in excess of the carbon dioxide equivalent of the sugar consumed in the same period. The three exceptions are the $\frac{1}{30}$ N treatment (shaded and unshaded) and the unshaded complete treatment of the potassium experiment. In these treatments the position is reversed and less carbon dioxide is evolved than the equivalent of sugar consumed. In the two treatments of the nitrogen experiment in which this occurred and in which the respiration rate increased during treatment there can be little doubt that the sugar unaccounted for was used in protein synthesis. In the remaining case where the respiration rate did not increase during treatment the fraction total nitrogen minus nitrate nitrogen did increase slightly, so there was some elaboration of nitrogen, but possibly not to the protein level, since no increase in respiration rate was observed.

In the instances where the CO_2 respired is in excess of carbohydrate consumed, a substrate other than carbohydrate must have been available. This most probably is protein or amino acids. Unfortunately only analyses of two nitrogen-fractions are available in the three main experiments, viz. total-N and nitrate-N, so that total-N minus nitrate-N is the best estimate available of protein-N. It is equally as good an estimate of soluble-N other than nitrate. The relationship between respiration rates and the total-N minus nitrate-N at the times of sampling, viz. 0, 2, 4, 8, 16 hours for all the data except the low nitrogen treatments, are shown in Fig. 8. There is a practically linear relationship between respiration rate and nitrogen content and nearly all the experiments lie fairly close together—in fact, the varying respiration rates of the several experiments can be brought into fairly good agreement. It is probable that the respiration rates are also partly dependent upon sugar concentration. It will be seen in Fig. 8 that two sets of points lie more remotely from the others. These are the values for the unshaded and shaded treatments for the low potassium ($\frac{1}{30}$ K) experiment. Here again the respiration is linearly related to the nitrogen content, but for a given rate of respiration the N content is higher than in the other treatments. It is suggested that this is because a potassium deficiency produces an excess of soluble N and protein synthesis is reduced. This has been observed in the cotton plant (Mason and Phillis, 1945), in wheat (McCalla and Woodford, 1935), and in the roots of pineapple (Sideris and Young, 1946), as well as in other plants. The literature bearing on this has recently been summarized by Street (1949).

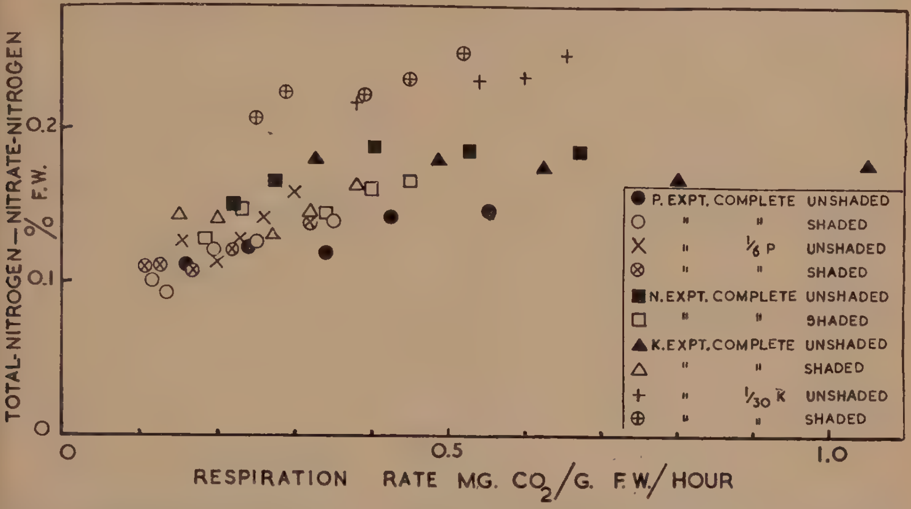

Fig. 8. The relation between the total nitrogen minus nitrate-nitrogen content and respiration rate of excised roots of barley plants grown under varying nutrient supplies when placed in an aerated complete culture solution.

In the preceding discussion no account has been taken of the varying sugar content with time because the sugar contents were not estimated at all sampling times, but considering the three experiments together there are 24 estimates of respiration rate, nitrogen content, and sugar content, namely the

0 and 16-hour values for each treatment in each experiment. From these data the partial regression of respiration rate on total-N minus nitrate-N is $+2\cdot53 \pm 0\cdot75$ and on total sugars is $+0\cdot28 \pm 0\cdot10$, indicating that respiration is dependent both on the nitrogen and sugar contents. Considering only the 0-hour values, the corresponding regression coefficients are $+2\cdot92 \pm 1\cdot14$ and $+0\cdot19 \pm 0\cdot12$, while using the 16-hour values the coefficients are $+0\cdot32 \pm 0\cdot50$ and $+0\cdot97 \pm 0\cdot17$. This suggests that at 0 hour sugar is relatively abundant and respiration is controlled by the nitrogen content, while at 16 hours the sugar content falls to a low level and then controls the respiration rate.

It is possible that the spread of the regression lines in Fig. 8 is due to the fact that protein and non-protein nitrogen have not been distinguished; had this been done the data from the various experiments might perhaps have tended towards a common regression line. There is, however, an evident relationship between nitrogen content and respiration rate of the excised roots. Whether this respiration is related to the protein content or a part of the soluble fraction such as amino acids it is not possible to say. It is interesting to note, however, that there is a considerable loss of nitrogen in most of the experiments where full nitrogen nutrition was provided, and it is probable that this is derived from the oxidation of the nitrogen compounds during respiration. Richards (1938) found with excised leaves of barley plants grown under a very wide range of nutritive conditions that there was a very constant relationship between respiration rate and protein content (at 25° C. it was approximately 0·165 g. CO_2 per g. protein per hr.) and suggested that there must be a reciprocal relation between protein and respiration rate, each being dependent in some measure on the other. He further suggested that to maintain a given protein level a certain minimal rate of CO_2 evolution may be necessary, and that if conditions are such that the existing protein cannot be maintained, hydrolysis sets in until protein is again proportional to CO_2 evolution.

In the subsidiary experiments described on p. 355 respiration rate as well as protein and non-protein nitrogen was measured at 0, 8, and 16 hours. The mean values for respiration rate are shown in Table IV together with the value of the nitrogen fractions. The respiration rate rose to a maximum at about 8 hours and then declined, while there was apparently no net protein synthesis during the latter half of the experiment. From these figures the mean value for the amount of CO_2 evolved, g./hr. per g. protein-N, at 0 hour is 0·326 and at 16 hours it is 0·340, while at 8 hours it is 0·562, this higher value being accounted for by the increase in CO_2 during protein synthesis. These data are admittedly rather fragmentary, but suggest that there is a fairly constant relationship between the respiration rate and amount of protein-N present.

VII. *Factors controlling gain or loss of ions*

In each of the experiments described one of the three ions, $H_2PO_4^-$, NO_3^-, or K^+, was supplied to some plants in only very small amounts, while the other two ions were supplied at a normal level. In each case there was an increase in the amount of the deficient ion when the excised roots were treated with a complete culture solution. In contrast, when an ion had been supplied in normal amount to the growing plant, the content of this ion is decreased in the excised roots under the same conditions. This suggests that one factor determining the gain or loss of a particular element is the concentration of that element present in the root cells; i.e. there appears to be an inverse relationship between rate of uptake of an element and the total concentration of that element in the roots at a given time. The data also indicate that ion uptake or loss is related to the carbohydrate content of the roots; when the carbohydrate content is high there tends to be absorption of ions, while when it is low a loss usually occurs. In order to investigate this statistically it was necessary to calculate the rates of loss or gain at the time when carbohydrate content had also been determined, i.e. at 0 hour and 16 hours. It is obvious from inspection of the observed data in Figs. 3, 4, and 6 that in most cases the regression of the content of a particular element with time is not linear, and this was confirmed by calculation of the quadratic term, which was in most cases significant. Hence quadratic regression lines were calculated for each independent replicate. The mean regression lines of the two replicates have been fitted to the data in Figs. 3, 4, and 6. From these curves the rates at 0 hour and 16 hours were computed.

The relation of the initial rate of uptake or loss of nitrogen to content of nitrogen is shown in Fig. 9 (*a*), and to total sugar content (sucrose+reducing sugars) in Fig. 9 (*b*). All the nitrogen data from the three experiments have been plotted. It is clear that there is a general inverse relationship between nitrogen content and rate; roots with a high nitrogen content tend to lose nitrogen, while those with a low nitrogen content gain. There is also a *direct* relationship between total sugar content and rate. With low sugar contents (below 0·2 per cent. of fresh weight) there is loss of nitrogen, and with higher contents gain of nitrogen occurs, the rate of gain increasing with increasing sugar content. The picture is similar for potassium and phosphorus.

Since uptake of an element appears to depend both on the content of that element and on the sugar content, the partial regressions of rate of uptake on total content and on total sugar content for each of the 3 elements in the 3 experiments have been calculated. These regression coefficients are set out in Table VII.

It will be seen that of the 9 partial regressions of initial rate on initial content 7 are significant. The regression for rate of uptake of total N on initial content in the potassium experiment failed to be significant, but in this experiment (p. 362) there was a rapid uptake of nitrate, some of which accumulated and was not assimilated. Calculation of the regression using the figures for non-nitrate N, i.e. total-N minus nitrate-N, gives a coefficient which is significant ($-36{\cdot}58 \pm 15{\cdot}17$).

The partial regressions of initial rate on initial total sugar content are significant in 5 cases out of 9, although one value is negative (phosphorus in the nitrogen experiment), which implies an increasing rate of uptake with a diminishing sugar content.

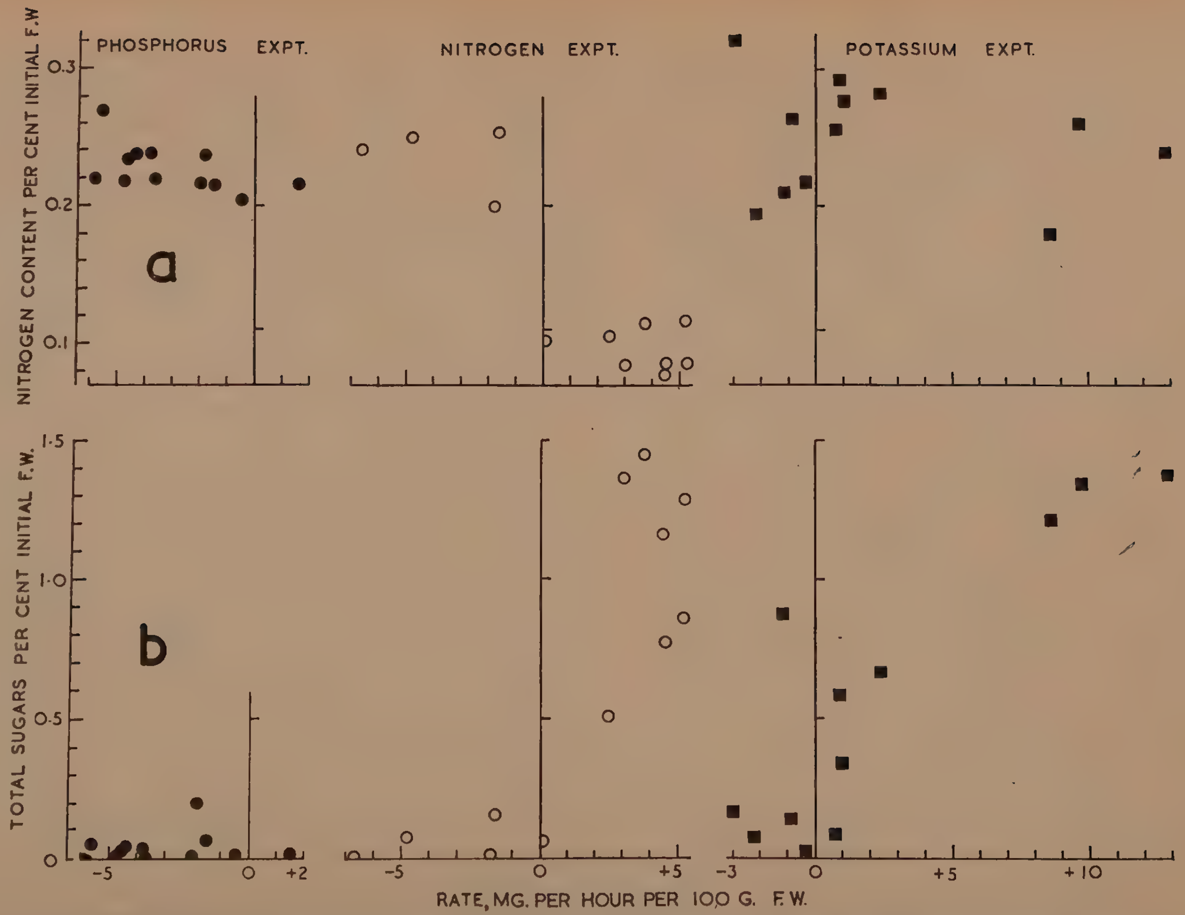

Fig. 9. The relation (*a*) between nitrogen content and rate of uptake of nitrogen, and (*b*) carbohydrate content and rate of uptake of nitrogen by excised barley roots in the phosphorus, nitrogen, and potassium experiments.

TABLE VII

Partial regression coefficients $(\times 10^3)$ *of rate of uptake of nitrogen, phosphorus, or potassium (mg./100 g./hr.) on content of nitrogen, phosphorus, or potassium and on total sugar content, mg. per 100 g., initially and after 16 hours treatment with a complete aerated culture solution; mg. per hr. per 100 g.*

	Nitrogen			Phosphorus			Potassium		
	N expt.	P expt.	K expt.	N expt.	P expt.	K expt.	N expt.	P expt.	K expt.
0 hour									
b_1 . . .	−37·14* ±11·42	−90·72* ±36·71	−2·95 ±20·40	−37·18* ±17·07	−47·88* ±8·89	−54·66* ±11·12	−103·55* ±27·06	27·52 ±25·79	−94·77* ±10·45
Total variance of rate of nutrient uptake on nutrient content, mg. per 100 g. F.W. per hr. $\times 10^6$	183·0	56·1	289·8	4·8	12·7	8·4	360·0	67·4	1465·2
Per cent. of variance accounted for by regression	81·4	27·9	70·5	47·5	79·2	73·2	60·1	0	95·2
b_2 . . .	2·37 ±1·41	15·20 ±11·53	8·55* ±1·66	−0·89* ±0·27	5·84* ±2·85	0·16 ±0·29	4·11* ±1·90	7·82 ±17·22	14·48* ±1·50
16 hours									
b_1 . . .	−23·37* ±10·70	−40·28 ±29·45	−19·20* ±4·72	−3·41 ±8·96	−15·10 ±13·14	7·04 ±6·34	−21·71 ±16·48	−91·46* ±21·60	−25·33* ±11·39
Total variance of rate of nutrient uptake on nutrient content, mg. per 100 g. F.W. per hr. $\times 10^6$	58·2	19·1	11·1	0·8	1·3	0·3	49·5	108·3	14·2
Per cent. of variance accounted for by regression	78·1	18·0	57·0	45·3	0	32·6	47·3	69·0	30·6
b_2 . . .	10·88* ±2·19	−148·79 ±88·46	2·85 ±1·79	1·21* ±0·46	−4·80 ±28·89	0·95* ±0·42	12·45* ±3·75	−155·43 ±139·42	6·06 ±4·85

* Indicates a significant regression coefficient.

b_1 = regression of rate of uptake of a nutrient (N, P, or K) on content of that nutrient.

b_2 = regression of rate of uptake of a nutrient (N, P, or K) on total sugar content.

The dependence of rate of uptake on content of element is not so striking when the 16-hour values are considered (4 significant out of a total of 9). In 4 cases also the partial regression of rate at 16 hours on the total sugar content at 16 hours is significant.

In Table VII the total variance of rate of uptake on nutrient content and the percentage of the variance accounted for by the regression are also tabulated. It will be seen that at 0 hour the percentage is greatest for nitrogen rate in the nitrogen experiment, for phosphorus rate in the phosphorus experiment, and potassium rate in the potassium experiment, i.e. most of the variance may be accounted for by the regression when the range of values of the particular nutrient is large. This is striking in the case of potassium in the potassium experiment where the regression accounts for 95 per cent. of the variance. It therefore appears likely that failure of some of the regression coefficients to attain significance is in part due to the restricted range of values over which the regressions are calculated. This might account for the smaller number of significant coefficients at 16 hours where the nutrient contents of the various treatments tend to even out and therefore exhibit a smaller range of values.

That the variance is smaller at 16 hours than at 0 hour is seen from Table VII, where each total variance of rate of nutrient uptake is set out. This is especially noticeable in the case of the potassium figures and suggests that potassium comes to an equilibrium sooner than the other two elements. It is also apparent from this table that the variance is greatest for the nutrient which had the greatest range of values, i.e. for the element which was most deficient in a particular experiment. An exception is the variance of nitrogen in the potassium experiment at 0 hour which is greater than the variance of nitrogen in the nitrogen experiment. This is possibly due to the fact that the plants in the potassium experiment became nitrogen deficient by depletion of the nutrient solution during growth and also because potassium deficiency had an effect on nitrogen content.

The partial regression lines for 0 hour and 16 hours in each experiment have been plotted, over the range of values encountered in the respective experiments, in Fig. 10. On the left-hand side of the graph the partial regressions of rate of uptake of a nutrient are plotted against content of that nutrient, while on the right-hand side the partial regressions of rate of uptake of a nutrient are plotted against total sugar content. Comparison of these graphs and the regression coefficients set out in Table VII serves to emphasize the point that where the range of any of the three nutrients and of sugar is large the coefficients are usually significant. These graphs also indicate that the partial regression coefficients of rate on sugar content tend to be larger with increasing mean sugar content. Where the range of nutrient content is large enough for the effect on rate of uptake to be detectable the slopes of the regression lines are similar. It should also be pointed out that in Fig. 10 the phosphorus values have been plotted on one-tenth of the scale of the other elements because of the smaller variation in phosphorus values, and this makes the regression lines more horizontal than those for nitrogen or potassium.

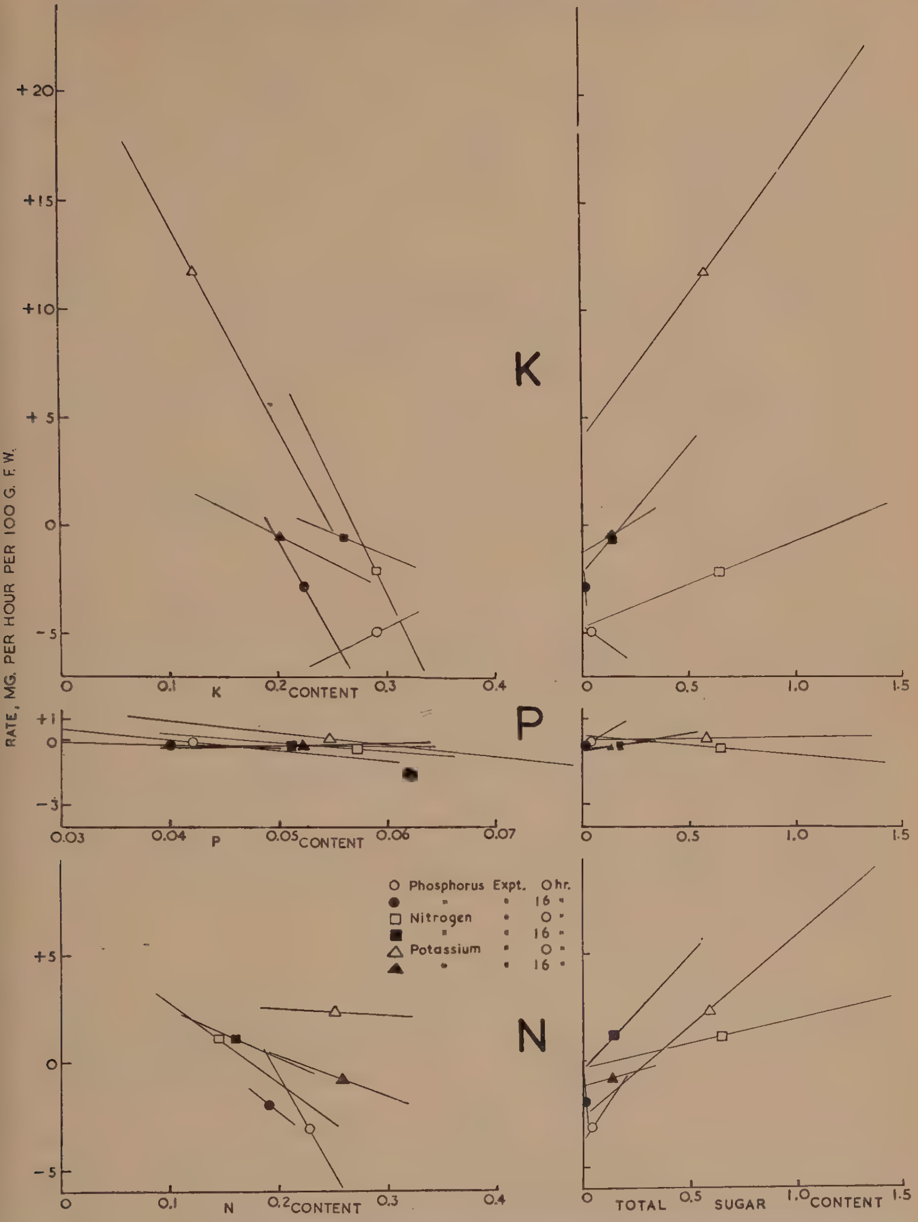

FIG. 10. The partial regressions of rate of uptake of potassium, phosphorus, and nitrogen by excised barley roots on potassium, phosphorus, or nitrogen content and on carbohydrate content.

This fact suggests that the rate of phosphorus uptake is not so dependent on phosphorus content as the rate of uptake of the other two elements are on the respective contents of those elements. That this is not the case is apparent from the regression coefficients set out in Table VII.

It may therefore be concluded that under the conditions imposed in these experiments the rate of loss or gain of phosphorus, nitrogen, or potassium at any time is determined by the content of phosphorus, nitrogen, or potassium at that time and by the total sugar content. Thus for a given content of any one element there is a critical concentration of sugar, above which the element will be absorbed by the root but below which loss of an element from the root will occur.

In the above analysis only 2-factor partial regressions have been considered, i.e. the regression of rate of uptake of, say, K on sugar content and K content which does not take into account the possible effect on K uptake of nitrogen or phosphorus contents. The regression analyses have been extended therefore to see if the rate of uptake of a given element is influenced by another. The method employed is that due to Cochran (1938), which permits the addition of an independent variate in a multiple linear regression and test of its significance without recalculation of all the regression coefficients. Thus, in the three experiments, 36 additional regression coefficients were calculated and in only 4 cases were significant values obtained, 3 of these being in the potassium experiment. The initial N and the initial K contents appeared to have an influence on the initial P rate, and the initial P rate had a significant effect on initial K rate. Apart from these three cases the only other significant effect was the 16-hour K content on the 16-hour N rate. It may therefore be concluded that in most cases the uptake of the three elements are virtually independent of one another. Independence of entry of the three elements is also supported by the fact that uptake of one element can occur simultaneously with loss of another. Alberda (1948), working with intact *Zea Mays*, found that plants grown under conditions of low phosphorus supply absorbed phosphorus at a rate twice as high as high-salt plants, and the rate was found to be independent of the concentration of other anions (NO_3^-, SO_4^-, Cl^-) in the nutrient solution.

VIII. *Changes in water content during treatment*

It has been shown in section II that there was a change in fresh weight and in water content of the excised roots during treatment, and that the extent of this change varied in the different experiments, there sometimes being a loss of water and sometimes a gain. A preliminary examination of the data suggested that this was connected with the carbohydrate status of the roots, but further examination shows that there is also a correlation between water gain or loss (subsequently referred to as the water-rate) and the uptake and loss of potassium. The water-rate for each set of data was calculated at 0 and 16 hours by the same procedure as adopted for the three elements and the partial regressions of this rate on sugar and K rate have been calculated. The partial regression coefficients are set out in Table VIII. It will be seen that at 0 hour and 16 hours in the phosphorus experiment and at 16 hours in the nitrogen experiment the partial regressions on sugar content are significant. In the phosphorus experiment and the potassium experiment there are indications that the water-rate is positively correlated with K rate, or, in other words, rate of water uptake is proportional to rate of potassium uptake. At 0 hour in the nitrogen experiment the water-rate was negatively correlated with K rate. Elimination also of nitrogen rate made no difference to these conclusions. There is evidence therefore that both sugar content and rate of potassium uptake have influence on rate of water uptake. Lundegårdh (1943) in discussing water movement into and out of cells points out that swelling of proteins is considerably augmented in the presence of certain cations. Ions of the alkali metals promote swelling but Ca causes the protoplasm to shrink. With the loss of one K ion the protoplasm must also lose some imbibed water which then accompanies the ion outwards, and he states that this amounts to 550 molecules of water for one K ion. If the roots are exposed to a calcium salt the loss of each K ion must imply a further predominance of Ca and a rapid decrease of the hydration of the protoplasm.

TABLE VIII

Partial regression coefficients of rate of water uptake by excised barley roots on total sugar content and on rate of potassium uptake

	Hours	Total sugars	K rate
Phosphorus experiment	0	2·31±1·14*	0·051±0·025*
	16	42·33±7·14*	0·088±0·009*
Nitrogen experiment	0	0·157±0·185	0·039±0·019*
	16	1·154±0·330*	0·002±0·024
Potassium experiment	0	0·131±0·277	0·021±0·012
	16	0·537±0·280	0·112±0·029*

* = significant regression coefficient.

Steward and Preston (1940) found that the presence of 0·005 M KBr or KNO_3 solution caused potato disks to gain more water than they did from distilled water, and they attributed this to some specific effect of the ions and not to an osmotic effect. They concluded that 'The presence of potassium promotes water absorption in potato discs in aerated solution, whereas presence of Ca depresses it'. There is therefore some support from the present series of experiments that potassium is to a certain extent concerned in the water balance of the cell.

DISCUSSION

There appear to be two main ways in which the uptake of the three elements described in this paper may be explained. The first is by a process of exchange and the second is by a process of accumulation. The first process involves no energy exchange while the second necessitates work being done in order to maintain a concentration within the cell greater than that of the external solution. When plants are deliberately grown with a deficiency of a cation such as potassium, other cations are usually taken up in excess, so that there is the well-known tendency for the total cations to be constant (e.g. Olsen, 1942). Under these conditions it might be expected that when the roots of such plants are treated with a complete nutrient solution, the deficient ion would tend to exchange with the cations previously taken up in excess. The possibility of this was investigated in the present experiments. After the main analyses had been completed calcium and sodium determinations were made on each sample by means of a flame-photometer. In the potassium experiment where the potassium-deficient plants were supplied with sodium it was found that in those cases where potassium increased during the course of treatment, calcium and sodium decreased, but in a much smaller equivalent amount than the increase in potassium. No determinations of magnesium (the other cation present) were made, but the total concentration and the changes in amount would have to be much greater than are at all probable to account for complete cation exchange. Thus the position seems to be that possibly a small amount of cation exchange takes place but this is insufficient to account for all the increase in the cation (K) observed.

It is usually assumed that most of the exchange which occurs between the cell and external solution is cationic and that exchange of anions is restricted because of the larger number of indiffusible anions (e.g. proteins) which cannot escape from the cell particularly in the cytoplasm (Robertson, 1941). This objection, however, may not be so strong in the present experiments, for it has been suggested that when nitrogen loss from the excised roots is occurring, proteolysis is taking place and that the loss of N occurs as some form of soluble nitrogen, which is very probably dissociated. Thus while some anion exchange is possible under the conditions of the experiments described, there is no evidence to determine the extent of this, and as the balance of cations cannot be explained on the basis of exchange, there is no reason to suppose that the anion balance can be more satisfactorily explained.

It has also been demonstrated that uptake or loss of an element is dependent on the level of sugar in the roots, there being a critical sugar content for any given content of element above which uptake occurs and below which loss occurs. This suggests that the absorption is governed by an energy-yielding process which affords additional evidence against an ion-exchange process being responsible for the changes observed.

As regards the alternative theory in the experiments described there was no evidence of a salt respiration during marked accumulation of potassium nitrate or phosphate, but an increase in respiration was evident when nitrogen was being absorbed and assimilated. It could be argued that, since there was a downward drift in respiration rate of the excised roots, that the respiratory substrate was insufficient to maintain an enhanced rate of respiration, and that if a salt respiration did occur in these experiments it would only be manifest as a less steeply falling respiration drift. This seems not to be the case since an increase in respiration rate did occur in the nitrogen experiments in which nitrate was absorbed and assimilated.

Robertson and Thorn (1945) point out that there has hitherto been no investigation on whether a balanced solution stimulates a salt respiration and suggest that roots of plants grown in culture solution may already have had a salt respiration induced by the culture solution, and the effect on respiration of placing such a tissue into a solution of another electrolyte would appear slight. This is a possible explanation of the absence of increased respiration observed in the phosphorus and potassium experiments, but is not consistent with the increase in respiration during uptake of nitrogen by nitrogen-deficient roots.

Thus it seems reasonable to conclude that a salt respiration did not occur in the phosphorus and potassium experiments although uptake of phosphorus and potassium was taking place. Lundegårdh and Burström (1933) in their early experiments on anion respiration found an increase in respiration rate during absorption of nitrate and other ions. Now it is clear that absorption of nitrate is a special case since if it is also at the same time assimilated a rise in respiration rate may be expected owing to the nature of the nitrate reduction which precedes protein formation. This rise in respiration rate due to absorption and subsequent utilization of nitrate cannot be termed salt respiration in the sense used by Robertson (1941), or anion respiration as used by Lundegårdh (1940), where an increase in respiration is envisaged as being a result of salt accumulation. Hence it appears that any experiments purporting to show a salt respiration when nitrate is the anion being accumulated must be suspect. The question then arises, in those cases where salts other than nitrates are being accumulated and an increase in respiration rate occurs, whether this increase is due to accumulation or the effect of the anion or cation or both in increasing protein synthesis. The latter is essentially the view taken up by Steward, Stout, and Preston (1940) and Steward and Preston (1941), who presented evidence in the case of potato disks to show that there was a parallelism between the effects on respiration and protein synthesis of the various treatments to which the disks were submitted. Thus protein synthesis occurred in disks immersed in potassium bromide, potassium nitrate, and calcium bromide, and the relationship between the total respiration after 70 hours and the amount of soluble nitrogen converted to protein was approximately linear. It was found, moreover, that there was a large part of the total respiration of potato disks which did not appear to be linked with protein metabolism. This is the respiration at zero protein synthesis or the component of CO_2 production which is not affected by salt and oxygen treatment and is not concerned in salt accumulation. They found that this value corresponded closely with the rate in distilled water. Thus Steward's conclusion that the respiration rate may be divided into two components does not conflict with the view of Lundegårdh and of Robertson on salt respiration, but the latter maintain that the accumulation of ions which takes place in the living cell is a function of the energy available from the extra respiration which the salt itself induces, whereas Steward would hold that the uptake of salt is related to the protein synthesis occurring. It seems impossible to decide which of these views is the more probable until more evidence is available. As Robertson himself points out, 'so far no investigations on carrot tissue have been made to determine whether the salt respiration is associated with oxidation processes in carbohydrate respiration as was suggested by Lundegårdh or with the protein synthetic mechanisms as Steward suggests'. In later work Robertson and Wilkins (1948) have found further evidence which seems to support the Lundegårdh hypothesis, and they have further elaborated it and postulated an energy-coupled system such as the cytochrome oxidase system. Lundegårdh (1951) has recently demonstrated the presence of cytochrome in living wheat roots. However, it seems fair to say that until experiments comparable with those done on potato disks have been done on carrot tissue, Steward's hypothesis cannot be ruled out. It could be surmised that the prolonged washing treatment to which carrot tissue is subjected before salt uptake experiments begin is conductive to proteolysis with a consequent increase in soluble nitrogen compounds at the expense of protein and that when a salt (e.g. KCl) is supplied the process is reversed and protein synthesis occurs and respiration rate increases. Certainly it has been shown that the length of the washing pre-treatment has a profound effect on the subsequent behaviour of the tissue; thus Steward, Berry, Preston, and Ramamurti (1943) found that washing potato disks in tap-water caused loss of potassium and possibly other solutes. They suggested that such a loss of solute engendered a deficit of total solute analogous to that produced during the growth of plants under low salt supply. They demonstrated that a longer washing time produced a greater intake of bromide in a given time. This enhanced absorption of ions by prolonged washing has been demonstrated by other workers with storage tissue, e.g. Stiles and Dent (1946), Asprey (1937), Rees (1949). Stiles and Dent found that the longer the preliminary washing in tap-water the sooner the second phase of absorption (equal accumulation of anions and cations) set in. These authors also found that extended washing in aerated running tap-water increased the respiration rate, and they suggest 'that development of the capacity for salt absorption goes along with the development of respiratory activity, i.e. enhanced salt absorption is related to increased metabolic activity in general and so to what Lundegårdh and Robertson have called ground respiration and not to the increment in salt respiration which results from the presence of salt'.

In the present experiments there is no evidence of a salt respiration in the phosphorus experiment although phosphate did accumulate in the low phosphorus roots. This apparently took place at the same time that nitrogen was being lost as a result, it is maintained, of protein hydrolysis. That is to say, there was definitely no net protein synthesis. Thus in the absence of a salt respiration or protein synthesis accumulation of phosphorus occurred. Similar results were obtained in the potassium experiments. These observations do not appear to fall into line with either of the two theories outlined above. Moreover, it does not appear that accumulation of a salt (i.e. equal accumulation of cation and anion) occurred in any of these experiments. In each of the experiments the deficient element was taken up in excess of the quantity of the other two ions which were estimated. Thus in the low phosphorus treatment there was a gain of 0·27 mg. eq. of phosphorus per 100 g. fresh wt. during the first 8 hours and a net loss of 0·09 mg. eq. of nitrogen and 0·124 mg. eq. of potassium. In the low nitrogen treatment, in a similar time, the roots gained 0·075 mg. eq. of nitrogen and lost 0·007 mg. eq. of phosphorus and 0·34 mg. eq. of potassium. In the low potassium experiment there was a gain of 3·82 mg. eq. of potassium and 1·3 mg. eq. of nitrogen and practically no change in phosphorus. In other words, in these experiments, if an element is deficient in the roots when exposed to a complete culture solution, that element is taken up in excess of its accompanying anion or cation. As previously stated, very little evidence was found for the uptake of one ion affecting the uptake of another by the excised roots. Uptake of potassium, for instance, is virtually independent of nitrate. The uptake of ions in these experiments appears to be analogous to a process of diffusion in that when a 'sink' for an element is created by the plants being grown in a deficiency of that element, it enters the root independently of other elements. A diffusion process, however, implies that ions move from a region of high concentration to a low, but the opposite is usually the case with living cells.

When loss of solutes from the excised roots occurred, as was frequently the case in these experiments, it might be argued that most, if not all, of the ions were lost from the cut ends of the roots by the well-known pumping action of roots. This does not seem to be very probable, for the following reasons. It has been shown that the rates of uptake and loss can be fitted on the same regression line, which suggests that both uptake and loss must occur by the same path, viz. through the root-cell membranes; otherwise it would have to be supposed that uptake takes place through the cells and loss through the vascular system at the point of severance. In these circumstances it must be postulated that at a given internal concentration of an element, as the sugar content falls and uptake gives place to a loss, that the pumping mechanism comes into play at a critical concentration of sugar and exudation occurs through the vascular system at an *increasing rate* as the sugar content falls. Alternatively it may be supposed that the pumping mechanism is in action all the time, but at a high sugar concentration that absorption by the root cells is in excess of exudation. When the sugar concentration falls below the critical level, exudation exceeds absorption. Here again it would follow that the rate of exudation increases with decreasing sugar content. It is usually assumed that the pumping action of roots is dependent on a high sugar content. Hence neither of the alternatives outlined seem very probable and it seems reasonable to maintain that the absorption and loss recorded in this paper takes place through the root cells and that loss by any other means is negligible. This contention is also supported by the observations of Lundegårdh (1943), who analysed exuded sap from wheat seedlings and found no sugar, protein, or nucleic acids and very little organic matter at all. It has been shown in the present investigation that nitrogen is lost in some organic form, and if the behaviour of barley is similar to that of wheat, it is probably exuded from cells and not from vessels.

ACKNOWLEDGEMENTS

My thanks are due to Dr. D. J. Watson for continued help and advice and to Miss B. Messer and Mr. S. A. W. French for technical assistance.

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